Université de Montréal

Biodiversity of Arbuscular Mycorrhizal Fungi from Extreme Petroleum Hydrocarbon Contaminated Site

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Mémoire présenté à la Faculté des études supérieures

en vue de l'obtention du grade de
Maître ès sciences (M. Sc.)
en Sciences Biologiques

août 2016

Université de Montréal
Faculté des études supérieures

Ce mémoire intitulé :

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a été évalué par un jury composé des personnes suivantes :

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Résumé

Les activités industrielles, la production d'énergie le transport et l'urbanisation ont engendré de sérieux problèmes environnementaux qui ont des effets néfastes non seulement pour les divers écosystèmes, mais aussi pour la santé des Humains. Il existe plusieurs méthodes de réhabilitation des sites contaminés. Les méthodes dites conventionnelles consistent le plus souvent à excaver, transporter et entreposer des sols dans des sites d'enfouissements, alors que d'autres technologies utilisent des traitements physico-chimiques ou l'incinération des polluants. Les inconvénients majeur de ces méthodes en sont le coût élevé, l'émission des gaz à effet de serre et la destruction des habitats. Cependant, plusieurs technologies ont émergé ces dernières décennies. Parmi ces technologies émergentes, la phytoremédiation est une méthode prometteuse et dont l'efficacité devienne de plus en plus reconnue. La phytoremédiation consiste à utiliser des plantes et les microbes qui leurs sont associés pour dégrader, extraire ou stabiliser les polluants du sol aussi bien organiques qu'inorganiques. Parmi les microbes associés aux racines des plantes, on trouve les champignons mycorhiziens arbusculaires (CMA) dont le rôle en phytoremédiation a été montré. Cependant, la diversité et les changements des structures des communautés de ces champignons dans des sites hautement contaminés et en association avec les populations des plantes qui poussent spontanément dans ces sites demeurent méconnues. L'objectif de mon projet de maitrise consiste à étudier la diversité et la structure des communautés des CMA dans les racines et les sols rhizosphériques de trois espèces de plantes *Eleocharis elliptica*, *Populus tremuloides* et *Persicaria maculosa* qui poussent spontanément dans des bassins d'une ancienne raffinerie pétro-chimique. J'ai échantillonné trois individus par espèce de plante dans trois bassins qui ont montré des concentrations différentes des polluants pétroliers. J'ai utilisé l'approche de la PCR conventionnelle, le clonage et le séquençage en ciblant le gène 18S de l'ARN ribosomique autant sur des échantillons de racines et des que sur ceux de sols rhizophériques. J'ai analysé au minimum 48 clones par échantillon. L'analyse de la diversité Beta a montré que la structure des communautés des CMA était significativement différente selon les biotopes (racines et sols rhizosphèriques) et les concentrations de contaminants pétroliers. Mes résultats ont montré que l'identité de la plante et la concentration de contaminants ont fortement influencé la structure des communautés de CMA. J'ai aussi observé qu'en plus de l'effet des facteurs biotiques et

abiotiques mentionnés ci-dessus, plusieurs OTUs de CMA sont corrélés soit positivement ou négativement entre eux et aussi avec différents types de polluants d'hydrocarbures pétroliers. Cette étude a permis de comprendre les facteurs qui influencent les changements des structures des communautés des CMA et pourrait nous aider à améliorer l'efficacité de la phytoremédiation avec des plantes indigènes poussant spontanément sur des sites hautement contaminés par des hydrocarbures pétroliers.

Mots clés: contaminants d'hydrocarbures pétroliers, phytoremédiation, champignons mycorhiziens arbusculaires, structure de la communauté, biodivertsité, PCR, clonage et séquençage.

Abstract

Industrial activities, energy production, transportation, and urbanization have led to serious environmental problems that have negative effects not only for the natural ecosystems, but also for the human health. Several methods of rehabilitation of contaminated sites such as conventional methods consisting on excavation, transportation and storage of contaminated soils in landfills (known as *Dig and Dump*), as well as other technologies that use physical and chemical treatments or incineration of polluted soil pollutants, have been largely utilized. However, these methods are very costly and not environmental-friendly because of greenhouse gas emission and destruction of habitats. Several green technologies have emerged in recent decades. Among these emerging technologies, phytoremediation is a promising method whose effectiveness becomes increasingly recognized worldwide.

Phytoremediation uses plant and their associated microbes to degrade, uptake or sequestrate organic and inorganic pollutants. Arbuscular mycorrhizal fungi (AMF) are among microbes that live intimately with plant root where they form a symbiosis known as arbuscular mycorrhiza. The objective of my master project was to study the diversity and changes of community structure of AMF in roots and rhizospheric soils of three native plant species *Eleocharis elliptica, Populus tremuloides* and *Persicaria maculosa* growing in petroleum-contaminated sedimentation basins of a former petro-chemical plant. I used conventional PCR, cloning and sequencing approach targeting 18S rRNA gene to investigate AMF community structure. I analyzed at minimum 48 clones for each sample. Beta diversity analyses showed that AMF community structure was significantly different across biotopes (roots and rhizospheric soils) and different concentrations of petroleum hydrocarbon contamination. Our results showed that plant identity and concentrations of petroleum hydrocarbon contaminations strongly influenced the AMF community structure as well as the inter-specific relationship among AMF taxa. Moreover, with consideration of both biotic and abiotic factors, we found that several AMF OTUs showed positive and negative correlations between each other and also with petroleum hydrocarbon pollutants. My study brings us in-valuable information to apply AMF for the phytoremediation in the future.

Key words: petroleum hydrocarbon contamination, phytoremediation, arbuscular mycorrhizal fungi (AMF), community structure, Biodiversity, PCR, cloning and sequencing.

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List of Abbreviations

PHC: petroleum hydrocarbon

AH: aliphatic hydrocarbons

PAH: polycyclic aromatic hydrocarbons

PCB: polychlorinated biphenyls

AMF: arbuscular mycorrhizal fungi

TPH: total petroleum hydrocarbon

Cd: cadmium

Cu: copper

As: ascenic

Cr: chromium

Zn: zinc

Pb: lead

Ni: nickel

P: phosphorus

N: nitrogen

HM: heavy metal

Al: alchemy

Mn: manganese

PCR: polymerase chain reaction

DGGE: denaturing gradient gel electrophoresis

TGGE: temperature gradient gel electrophoresis

rRNA: ribosomal ribonucleic acid

OTU: operational taxonomic unit

DNA: deoxyribonucleic acid

LC: low contamination

MC: moderate contamination

HC: high contamination

PCoA: principal coordinate analysis

CCA: canonical correspondence analysis

HCA: hierarchical clustering analysis

PPN: plant-parasitic nematode

Acknowledgements

I am glad to spend a wonderful time during my work and study in the biodiversity center. I would like to thank the people in the center who have helped me to carry out this work.

I would like to show my thanks to Biodiversity center, IRBV, Université de Montréal, for providing me the facilities for accomplish this research.

I would like to thank my director professor Mohamed Hijri who always helps and encourages me in my study and research.

I would like to thank all the members of jury, Prof. Marc St-Arnaud, Prof. Mohamed Hijri and Prof. Étienne Laliberté who correct and commend my thesis.

I would like to thank my parents and friends; I appreciate their kindness and patience to my study.

Chapter 1

General introduction

Petroleum hydrocarbons (PHC) are the most widespread oil contaminants all over the world, and it consists of a large range of the organic compounds including: aliphatic hydrocarbons (AH), polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB), which can cause a wide variety of problems related to their toxicity, mobility and persistence, especially the toxicity to human, animal, plants and environmental health. Conventional methods to cleanup petroleum hydrocarbon polluted sites relay on the use of physico-chemical treatments or excavation, transportation and storage. All these methods are very costly and inappropriate for large contaminated areas. In addition, these methods contribute to greenhouse gas emission because of the use of heavy machinery and destroy soil habitats. Alternative methods which are economical affordable and environmental friendly have been developed and utilized in the past decades. Among these methods, phytoremediation, which uses plants and their associated microbes to absorb, degrade, mitigate the pollutants *in situ* .

1.1: Petroleum Hydrocarbon contamination

1.1.1: What is petroleum hydrocarbon contamination?

Petroleum hydrocarbon is a large range of compounds resulting from the processing of the crude oil. The modern petroleum industry started with a man named Edwin L. Drakein, who constructed the first oil well to extract petroleum from the natural oil in Pennsylvania, 1859. Since then, the oil industry has become the most profitable and fastest growing business in the world.

The rapid growth of the oil industry, while promoting the progress of human society, but also caused incalculable damage and pollution to the natural environment. Apart from the refining derivatives, the oil spills caused catastrophic oil product leaking to the environment, which increased the severity of the pollution. Here is the list of the thirteen largest oil spills in history by volume (Moss 2010) (Table1).

Table 3 List of the thirteen largest oil spills in history by volume (Moss 2010).

Location	Date	Amount spilled
1. Arabian Gulf/Kuwait, Persian Gulf, Kuwait	Jan. 19, 1991	380-520 million gallons
2. Gulf oil spill, Gulf of Mexico	April 22, 2010	An estimated 206 million gallons
3. Ixtoc 1 Oil Spill, Bay of Campeche off Ciudad del Carmen, Mexico	June 3, 1979	140 million gallons
4. Atlantic Empress Oil Spill, Off the coast of Trinidad and Tobago	July 19, 1979	90 million gallons
5. Kolva River Oil Spill, Kolva River, Russia	Aug. 6, 1983	84 million gallons
6. Nowruz Oil Field Spill, Persian Gulf, Iran	Feb. 10, 1983	80 million gallons
7. Castillo de Bellver Oil Spill, Saldanha Bay, South Africa	Aug. 6, 1983	79 million gallons
8. Amoco Cadiz Oil Spill, Portsall, France	March 16, 1978	69 million gallons
9. ABT Summer Oil Spill, About 700 nautical miles off the coast of Angola	May 28, 1991	51-81 million gallons
10. M/T Haven Tanker Oil Spill, Genoa, Italy	April 11, 1991	45 million gallons
11. Odyssey Oil Spill, Off the coast of Nova Scotia, Canada	Nov. 10, 1988	40.7 million gallons
12. The Sea Star Oil Spill, Gulf of Oman	Dec. 19, 1972	35.3 million gallons
13.The Torrey Canyon Oil Spill, Scilly Isles, U.K.	March 18, 1967	25-36 million gallons

These petroleum wastes are highly toxic to the environment especially to human beings when in a large concentration. The direct contact with petroleum pollutants including skin absorbing and breathing can cause mild to sever illness even cancer, and the indirect contacts such as water and food contamination can also increase the health risk. Some research showed the exposures to the spilled crude oil were associated with significant increases in the period prevalence for diarrhea, sore eyes, itchy skin and occupational injuries (Ordinioha and Sawyer 2010)(Table2). Coincidentally, some animal studies which use food contaminated with crude oil to feed rats and other experimental animals showed sever impact on animal fertility (Adesanya, Shittu et al. 2009).The research based on the plant health under crude oil contamination indicated the oil pollutants could reduce the photosynthesis though the disruption of the chloroplast membranes and inhibition caused by accumulation of end-products (Baker 1970). Moreover, some plants can be directly killed by spraying light oil on young tissues when their stomata are open (Baker 1970).

Table 4 Symptoms reported by respondents by exposure categories and associations (Ordinioha and Sawyer 2010).

Variable	Exposed(%)(N=210)	Unedxposed(%)(N=210)	O/R	P value
Malaise	49(23.33)	33(15.77)	1.63	<0.05
Headache	76(36.19)	27(12.86)	3.84	<0.001
Nausea	48(22.86)	11(5.24)	5.36	<0.001
Diarrhoea	87(41.43)	28(13.33)	4.6	<0.001
Sore eyes	69(32.86)	9(4.29)	10.93	<0.001
Sore throat	63(30)	13(6.19)	6.49	<0.001
Cough	56(26.67)	17(8.1)	4.13	<0.001
Itchy skin	103(49.05)	14(6.67)	13.48	<0.001
Rashes	90(42.86)	13(6.19)	11.37	<0.001
Occupational Injuries	51(24.29)	12(5.17)	5.29	<0.001

The petroleum pollutants have a large range of chemicals involved, such as hexane, benzene, toluene, xylenes, naphthalene, fluorine, gasoline, jet fuels, mineral oils, and other petroleum products. According to their chemical characteristics, they can be generally separated into three main groups: aliphatic hydrocarbons (AH), polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB).

1.1.2: Petroleum hydrocarbon compounds

Aliphatic hydrocarbons (AH) are known as non-aromatic compounds, the carbons are joined by open chains (single bonds, double bonds and triple bonds). Most of the aliphatic hydrocarbons are volatile and flammable, normally to be used as fuel and gas such as methane and acetylene. The toxicity and potential danger of the AH is related with their high carcinogenicity and flamability. Some study showed that parafins and olefins can cause irritation when applied to the intact skin, and some animal experiments and clinical experiences indicate that with the higher olefins, an injurious effect on the liver and a vagomimetic action may be observed (Reynolds and Moslen 1977).

Polycyclic aromatic hydrocarbons (PAH) are a group of petroleum compounds composed of multiple aromatic rings, like phenanthrene and anthracene, which represent the starting members of the PAHs. They normally form a large portion of the TPH (total petroleum hydrocarbon). The

PAH which contains less than six aromatic rings are more often considered as small molecule PAH, and the one which contain more than six aromatic rings are considered as large molecule PAH. Compared to the small molecule PAH, the large molecule PAH are more persistent and hard to degrade. Moreover, most of the recent research on the PAH is based on the small molecule PAH. PAHs are toxic, mutagenic and/or carcinogenic. They are insoluble in water and soluble in lipids and many organic agents. Due to this chemical characteristic, they can be easily absorbed by cells and stored in lipids, which could pose a threat to human health. In general, the more heavier PAHs are, the more carcinogenic they tends to be, for example, the PAHs containing four, five or six rings are more toxic than the PAHs contains three rings (such as the 3-ringed phenanthrene) (Irwin 1997). Some animal study showed oral exposure and dermal exposure to the PAH can cause systemic effects, immunological effects, reproductive effects and cancer of mammals (Robertson and Hansen 1947).

Polychlorinated biphenyls (PCB) are organic chlorine compounds with the formula $C12H10{-}xClx$ (Rossberg, Lendle et al. 2006). They are found in the petroleum wastes and also commercially used as an additive in pesticides, paints and carbonless copy products. PCB are quite persistent in the environment, where they can be degraded by some bacteria and eukaryotes, but the speed is normally very slow and they can accumulate in plants, animals and human body (Robertson and Hansen 1947). PCB have been proved toxic and carcinogenic, some study showing that women exposed to PCB before or during her pregnancy can result in giving birth to a child with lowered cognitive ability, immune compromise, and motor control problems (Jacobson and Jacobson 1996, Stewart, Reihman et al. 2000). In addition, other research indicated PCB can interferer with estradiol in human body, which lead to a toxic and mutagenic effect. Therefore, the disposal of PCB can cause serious environmental and health concern (Layton, Sanseverino et al. 2002).

1.2: Phytoremediation

Phytoremediation is a plant-based technique, which use plant as well as its rhizospheric microbes to clean, degrade, restore, transfer, transform and remove the possible pollutants in soil (Reichenauer and Germida 2008). In the recent year, this technique has received more and more attention as a cost-effective, environmental friendly, innovative and easily applicable treatment

method (Susarla, Medina et al. 2002, Pulford and Watson 2003). There are some remediation techniques belonging to the concept of phytoremediation such as: phytoextraction (Jadia and Fulekar 2009), phytostabilization, phytotransformation, phytovolatilization, rhizofiltration and phytodegradation (Long, Yang et al. 2002) (Figure1).

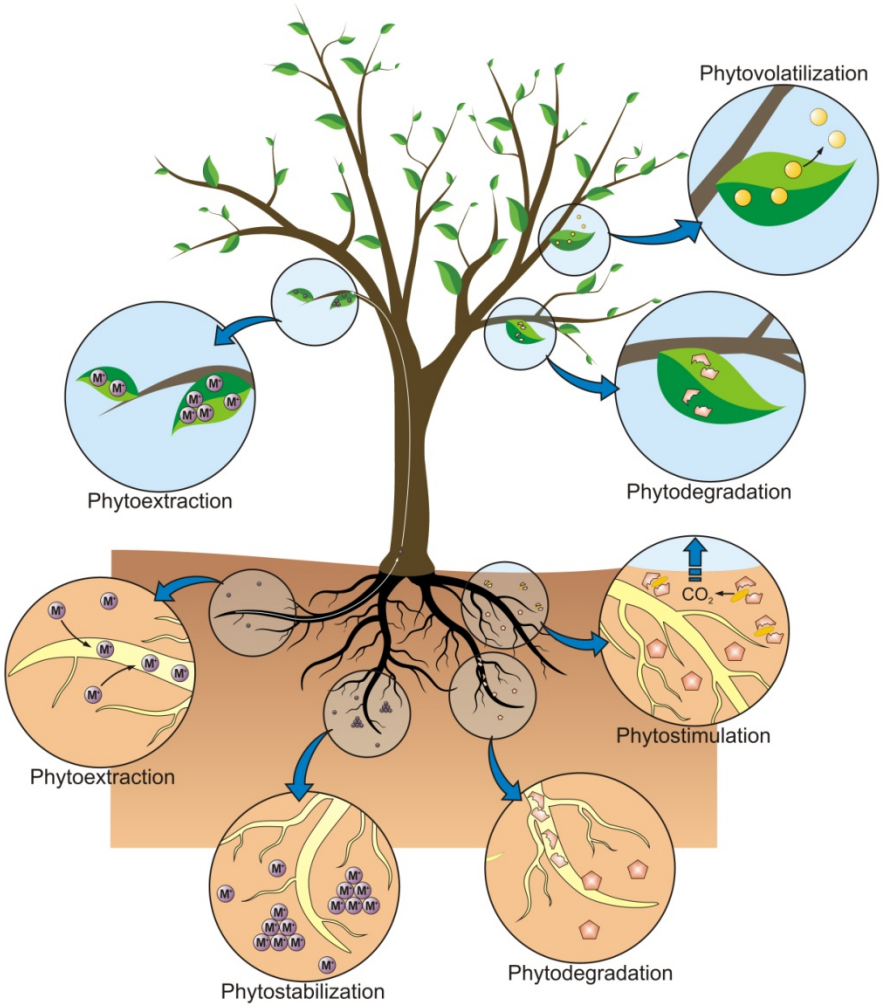

Figure 1. Remediation techniques belonging to the concept of the phytoremediation (Favas 2014).

- In Phytoextraction, plant roots can absorb pollutants from the soil and transfer them to the plant shoots or accumulate them in the roots. The pollutants can be then removed by harvesting the roots and shoots of the plant (Salt, Blaylock et al. 1995). This approach was mostly utilized to clean up trace elements contamination (Jiang, Yang et al. 2004, Zhang, Dang et al. 2009). Some reports showed that the cost of phytoextraction can be up to ten

times cheaper compared with the conventional soil remediation methods (Borisev, Pajevic et al. 2009), and it's highly recommended for large areas of mining sites.

- Phytostabilization means the stabilization of pollutants by plants, which can limit and prevent the leaching of the pollutants into underground water (Raskin and Ensley 1999). Some reports showed that phytostabilization is an effective technique for the remediation of some trace elements such as Cu, Cd, As, Cr, Zn (Alvarenga, Goncalves et al. 2009).
- Phytotransformation means the use of plant and its rhizospheric microbes to modify, metabolize the pollutants, to reduce or remove the toxicity of the pollutants. It differs from phytodegradation. Phytotransformation may not break the chemicals into small molecules, like amino acids or proteins, it only transform the chemicals from one form to another.
- Phytovolatilization uses plants to uptake pollutants from soils, degrade and volatilize these pollutants into the air by plant transpiration. This method is mostly used for the pollutants such as organic compounds and some trace elements such as Hg and Se (Karami and Shamsuddin 2010).
- Rhizofiltration uses wetland plant roots systems to filter toxic substances from the water. This method is mostly used for Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily retained within the roots (United States Protection Agency, 2000), and nitrates.
- Phytodegradation uses plant roots and rhizospheric microbes that release enzymes produced by both plants and microbes to degrade, metabolize, or detoxify contaminants (Garbisu and Alkorta 2001). This method is similar to phytotransformation, which can break down some complex chemicals in to smaller molecules (Prasad and Freitas 2003).

The advantages of phytoremediation are:

- Easy operation, no heavy machinery needed, no chemical processing required.
- Green technology, environmental friendly, *in situ* treatment and suitable for large area.
- Can be used for both organic and inorganic pollutants.
- Low operating cost compared with conventional methods.

The disadvantages of phytoremediation are:

- Slow process that requires a long period of time.

- Unpredictable results.
- The use of introduced plant species may affect the biodiversity of the ecosystem.

Phytoremediation continues to improve its efficiency and it benefits greatly from advances made in next generation sequencing. In some cases, phytoremediation is considered as the unique approach. Some authors showed that phytoremediation can be efficient in a short period of time (90 days) in removing totally or partially organic compounds from sediment (Jones, Sun et al. 2004, Moreira, Oliveira et al. 2011).

1.3: Role of Arbscular Mycorrhizal Fungi in phytoremendiation

Arbuscular Mycorrhizal Fungi (AMF) are among the most important fungal-plant symbionts on earth. They are able to form mutualistic symbiosis with more than 80% of plants in terrestrial and aquatic ecosystems (Brundrett 1991, Azaizeh, Marschner et al. 1995). AMF hyphae colonize the cortex of roots where they can penetrate the cortical cell and form highly branched structures, called "arbuscules". Arbuscules are considered to be an active interface for nutrients exchanges between plants and AMF. It has been largely documented that AMF improve plant nutrients uptake, particularly phosphorus (P), and also other macro- and oligoelements (Smith and Read 2008, Roy-Bolduc and Hijri 2011, Hijri 2016). As an obligate symbiont, AMF require to live with autotrophic plants to complete their life cycle due to their limited capacity for utilizing carbon source (Zhang, Xu et al. 2016). A tradeoff of carbon and phosphorus occurs between the partners through arbuscules. Many studies have demonstrated that AMF enhanced plant tolerance against biotic and abiotic stresses such as: nutrient limitation, salinity, drought, accumulation of trace metals, pesticides, and petroleum hydrocarbon pollutants, plant-parasitic nematode (PPN) infection (Davies, Potter et al. 1993, Estrada-Luna, Davies Jr et al. 2000, Davies, Puryear et al. 2002, Amaya-Carpio L. 2005, Schouteden, De Waele et al. 2015). The increased tolerance of plants could be explained by an improved nutrients uptake as well as induced defense mechanisms. Lee and George (2005) found that inoculation with AMF could reduce the Cd and Ni transfer from plant roots to shoots. Wu, Yu et al. (2014) showed that *G. mosseae* significantly increased ryegrass growth under PAH contamination by enhancing photosynthetic activity through increasing the chlorophyll content in shoot. Yu, Wu et al. (2011) observed a higher dissipation rates of PAH when ryegrass were inoculated with *G. mosseae*. AMF has been

considered as one of the most important soil microbes in phytoremediation, although their direct role on degradation of organic pollutants remains unclear. Yang, Liang et al. (2016) have inoculated the legume tree *R. pseudoacacia* with the AMF *R. intraradices* in a phytoremediation experiment that was successfully established on a trace metal contaminated site. The results showed AMF symbiosis can reduce the Pb toxicity in *R. pseudoacacia* by decrease Pb concentrations in leaves, which suggested using two AMF species associated with *R. pseudoacacia* can be a good method for phytostabilization of Pb contaminated soils. Alori and Fawole (2012) also showed the potential utilization of *Scutellospora reticulata* and *Glomus pansihalos* to enhance phytoremediation of soils contaminated with Aluminum (Al) and Manganese (Mn).

1.4: Molecular methods to assess AMF diversity and community structure

Conventional methods to study AMF community structure in the field rely mostly on the morphology of spore such as color, shape, size, ornamentation, hyphal attachment, spore wall layers, etc. These methods are fastidious, time consuming and may lead to misidentification. They also require extensive expertise in taxonomy and microscopy. In addition, sporulation may be affected by many biotic and abiotic factors and some AMF taxa could not form spores. It has been also reported that sporulation of AMF can fluctuate with seasons, and varies between different host plant species. Environmental stress such as soil pollution or nutrients limitation can negatively impact AMF sporulation. Moreover, the sporulation of AMF species highly depends on its species. For example, *Rhizophagus irregularis* is one of the most commonly found AMF species in the nature due to its fast growth and sporulation. Some AMF families are barely observed with spores; instead, they just form mycelia most of the time.

In the last decade, the advances that have been made on next generation sequencing technologies allowed to make a considerable progress on molecular tools to study AMF community structure (Wilde, Manal et al. 2009, Hassan et al. 2011). Most of the molecular tools available to date rely on ribosomal DNA, although, other candidate genes have been utilized. PCR, denaturing gradient gel electrophoresis (DGGE), temporal temperature gradient gel electrophoresis (TGGE), restriction fragment length polymorphism (RFLP), and cloning-sequencing has been largely used to study AMF biodiversity and their ecology (P. Cornejo 2004, Ma, Siciliano et al. 2005, M. Zarei 2008, Z. Liang 2008, Sonjak, Beguiristain et al. 2009). The *Glomeromycota* specific primers

AML1 and AML2 designed on 18S small subunit (SSU) rDNA have been used to study AMF (lee and Young 2008). They can cover all AMF species and a 850 bp amplicon can be obtained. However, these primers can also amplify other fungal taxa such as *Ascomycetes* and even sometimes plants. Hassan et al. (2011) found that 40% of amplified sequences using AML1 and AML2 were *Ascomycetes*. High throughput sequencing such as 454 sequencing and Illumina sequencing were largely used for the ecological study of the diversity of soil microbes including AMF (Bell, Hassan et al. 2014) due to their low cost on large scale of samples (Colombo, Bidondo et al. 2014, Xiang, Verbruggen et al. 2014, Wu, Wen et al. 2015, Zhang, Qu et al. 2015, Zhang, Qu et al. 2015).

1.5 Objectives

Because of the important suspected role of arbuscular mycorrhizal fungi in phytoremediation both in organic and inorganic contaminated sites it is relevant to study the diversity and environmental factors that drive the changes of the community structure of AMF in spontaneously growing plants in polluted sites. The objective on my master project was to investigate the diversity of AMF and their community structure in a former sedimentation basins polluted with petroleum hydrocarbons in which spontaneous plants were growing. I used PCR, cloning and sequencing approaches on DNA extracted form rhizosphere soils and roots of three native plant species *Eleocharis elliptica, Populus tremuloides* and *Persicaria maculosa* to retrieve the community structure of arbuscular mycorrhizal fungi from the petroleum hydrocarbon contaminated sites.

The specific objectives were: to compare AMF richness in rhizospheric soils and roots of three host plants in different contamination concentrations; to assess the effect of contamination concentrations and biotopes on AMF community structure; to infer correlations between AMF taxa and chemical pollutants.

Chapter 2

Chapter 2 is presented as a research article which is in preparation for publication. I have made the major contribution of this study including sampling, processing of all samples, DNA extraction, PCR, cloning and sequencing. I have also made major bioinformatics as well as statistical analyses.

Petroleum hydrocarbon contamination and plant identity structured arbuscular mycorrhizal fungal communities in roots and rhizosphere of three native plant in extreme polluted environments

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Running title: AMF community structures shift in roots and rhizosphere

Keywords: Arbuscular mycorrhizal fungi, community structure, Petroleum-hydrocarbon contamination, PCR, cloning and sequencing, Ribosomal DNA

Abstract

Arbuscular Mycorrhizal Fungi (AMF) has been shown to play an important role in phytoremediation of trace elements and petroleum hydrocarbons contaminated soils, although their mechanisms involved in pollutant cleanup remain unclear. We studied community structure of AMF in roots and rhizospheric soils of three native plant species *Eleocharis elliptica, Populus tremuloides* and *Persicaria maculosa* growing in petroleum-contaminated sedimentation basins of a former petro-chemical plant. These sites showed extreme concentrations of pollutants including aliphatic hydrocarbons (AH), polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB). We sampled three individual plants for each plant species in three adjacent basins that have different concentrations of contaminants. We used a conventional PCR, cloning and sequencing approach targeting 18S rRNA gene to investigate AMF community structure. We

analyzed at minimum 48 clones for each sample. Beta diversity analyses showed that AMF community structure was significantly different across biotopes (roots and rhizospheric soils) and different concentrations of petroleum hydrocarbon contamination. We also showed that plant identity and concentrations of petroleum hydrocarbon contamination strongly influenced the AMF community structure as well as the inter-specific relationship among AMF taxa. Moreover, with consideration of both biotic and abiotic factors, we found that several AMF OTUs showed positive and negative correlations between each other and also with petroleum hydrocarbon pollutants.

2.1 Introduction

Arbuscular Mycorrhizal Fungi (AMF) has been considered one of the most important microbial groups in the plant roots and rhizosphere because of their ubiquity and their state of obligate symbiont with plants (Brundrett 1991, Azaizeh, Marschner et al. 1995). It is well documented that AMF improve plant growth by increasing their mineral uptake, in particular phosphorus (Smith and Read 2008, Roy-Bolduc and Hijri 2011, Hijri 2016). As a trade-off, AMF receive carbon from the plant (Smith and Barker 2002). In addition to plant nutrition, AMF protect plants against biotic and abiotic stresses. Many studies have showed that AMF enhanced plant survival from environmental stress such as: nutrient limitation, salinity, drought, trace elements, petroleum hydrocarbon pollutants, plant pathogens and plant-parasitic nematode (PPN) infection (Davies, Potter et al. 1993, Cabello 1999, Estrada-Luna, Davies et al. 2000, Davies, Puryear et al. 2002, Hassan, Hijri et al. 2013, St-Arnaud and Vujanovic 2007, Yang, Han et al. 2015). Evidences have been reported that AMF can impact the microbial community structure of soil microbes that enhance degradation, sequestration or stabilization of pollutants (Jeffries, Gianinazzi et al. 2003, Johansson, Paul et al. 2004, Barea, Pozo et al. 2005). Several studies also showed that AMF could improve phytoremediation to clean up trace metals polluted soil (Pawlowska, Chaney et al. 2000, Rivera-Becerril, Calantzis et al. 2002, Hassan et al. 2011, Hassan, Hijri et al. 2013). As obligate symbionts, AMF show host-plant dependency as well as a community structure specificity in different environmental condition (Eom, Hartnett et al. 2000, Torrecillas, Alguacil et al. 2012, Yang, Zang et al. 2012, Guo and Gong 2014). Some studies indicated that diversity of AMF could be impacted and modified by contamination such as trace elements and petroleum hydrocarbons

(Hassan et al. 2011, Hassan, Bell et al. 2014, de la Providencia, Stefani et al. 2015). This makes the application of AMF in the field challenging and unpredictable. Morover, studies on remediation of petroleum hydrocarbons by using AMF in large-scale trials are still limited. Therefore, it is essential to understand the diversity of AMF and their correlation with biotic and abiotic environmental factors in petroleum hydrocarbon contaminated sites.

The objectives of our study were (1) to investigate the diversity of AMF and their community changes associated with three native plants growing in extreme petroleum hydrocarbon polluted environments; (2) to evaluate the impact of different contamination concentrations on AMF community structure. We used PCR, cloning and Sanger sequencing based on 18S rRNA gene to amplify an approximately 750 bp fragment of AMF. We report evidences of a correlation between the AMF community and three distinct environmental factors: host plant species identity, biotopes (roots or soils), and contamination concentrations (low, moderate and high).

2.2 Material and methods

2.2.1 Site of study and sampling

Sampling was conducted in artificial sedimentation basins of a former petro-chemical plant located in Varennes, Montreal region, Quebec (45° 41′56″ N 73° 25′ 43″ W), where the petroleum hydrocarbon wastes were dumped for several decades. The plant was closed in 2008 and the basins were left for vegetation by spontaneous plants. Three adjacent basins were separated by dikes but were connected to each other (Figure S1). An exhaustive inventory of plants was done in one decantation basins (Desjardins, Nissim et al. 2014) where the authors reported that the site was characterized by a patchy revegetation dominated by *Eleocharis obtusa* and *Panicum capillare*. However, we choose to sample the following three plants species *Persicaria maculosa*, *Eleocharis elliptica*, *Populus tremuloides* which were found in the three basins although they were not dominant plants considering they are the only native plants present in all three basins. We collected three individuals for each plant species as well as their rhizospheric soils in each basin. Samples were put separately in plastic bags, stored in a cooler, and immediately transported to the lab. Taxonomic identification of plants has been conducted and specimens were deposited in the Marie-Victorin herbarium (biodiversity center, Université de Montréal). Roots were separated from rhizospheric soils, cleaned under tap water and stored at -80°C until further DNA

extraction. Rhizospheric soils were also stored at -80°C.

2.2.2 DNA extraction

Root DNA was extracted using the commercial DNeasy Plant Mini kit (QIAGEN) from 1 g of root samples crushed with liquid nitrogen using a mortar and pestle. Soil DNA was extracted from 500 mg soil samples using the PowerSoil DNA Isolation kit (MO BIO Laboratories). Both extractions series were performed following the manufacturer's instructions.

2.2.3 PCR, cloning and sequencing

PCR amplifications were individually performed on the DNA extracted from the root and soil samples using primer pair AML1 (5'-ATCAAC TTTCGATGGTAGGATAGA-3') and AML2 (5'-GAACCCAAACACTTTGGTTTCC-3') to amplify a 750 bp fragment of the 18S rRNA gene (Lee, Lee et al. 2008). PCR was performed by using the following cycling program: initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, and a final extension period at 72°C for 10 min. Lne µl of diluted (1/10) DNA was used as template for PCR reactions in a 50 µL volume containing: 1x PCR buffer, 1U of *Taq* DNA polymerase (Qiagen), 0.25 mM dNTP mixture, and 0.4 µM of each primer. PCR products were run on a 1% agarose gel electrophoresis, stained with GelRed, and visualized using a GelDoc imaging system（Bio-Rad). The PCR products were cloned using the CloneJET PCR Cloning kit (Thermo Fisher Scientific) following the manufacturer's instructions. Ligated plasmids were transformed into competent *E. coli* TOP10 cells (Thermo Fisher Scientific) using a heat-shock approach. The transformed bacteria were plated onto LB (Luria-Bertani) medium containing 100 µg/ml ampicillin. PCR using AML1 and AML2 primers was performed directly on bacterial colonies to screen positive clones. Clones that showed fragments with the expected size were sent for sequencing at the Mcgill University and Genome Québec Innovation Center (Montréal, QC).

2.2.4 Soil analyses

Composite soil samples were taken from 9 soil subsamples in each basin where plants were

sampled in July 19th, 2012. The soil of each basin was characterized by measuring petroleum hydrocarbons (alkane C10-C50, polycyclic aromatic hydrocarbons and polychlorinated biphenyls) (Maxxam Analytics, Montreal, Quebec, Canada). The concentrations of these petroleum hydrocarbons are shown in the Table S2. The total petroleum hydrocarbon concentrations of each basin were 3000 μg kg^{-1}, 41000 μg kg^{-1} and 91000 μg kg^{-1}, which was categorized respectively as Low contaminated (LC), moderate contaminated (MC) and highly contaminated (HC) sites.

2.2.5 Bioinformatics and statistical analyses

Bioinformatics and statistical analyses were conducted in Mothur pipeline (v.1.31.2) (Schloss, Westcott et al. 2009) and using the XLSTAT-Ecology software (Addinsoft, France), respectively. Sequences were aligned using the command ”align” with a cutoff of 0.03 in Mothur for SSU sequences belonging to the same operational taxonomic unit (OTU) to represent as a proxy for ‘species’, all the high variable and poorly aligned sequences were removed by using commands “screen” and “filter”. The “summary” of all sequences were obtained by the command “summary.seqs” (Table 1). Uncorrected pairwise distance between each sequence was conducted by command “dist.seqs”, cluster of the sequences was done using commands “clearcut” and “cluster”. Representative sequences from each OTU were generated by the command “get.oturep”. The consensus sequences were combined with the sequences from Kruger, Kruger et al. (2012) and with the closest sequences retrieved in MaarjAM database (Opik, Vanatoa et al. 2010).. Taxonomic assignments were performed at 97% of sequence similarity. Alignments were done using MUSCLE v.3.6 (Edgar 2004). The DNA substitution model was determined using the Bayesian information criterion calculations implemented in jModelTest v.2.1.7 (Darriba, Taboada et al. 2012). Bayesian phylogenetic analyses were performed as described in de la Providencia, Stefani et al. (2015) with 20,000 generations of trees and the first 3,000 trees were removed. Rarefaction curves were conducted in R (version 3.3.2) by command “rarecurve” in “vegan package” and diversity calculation were generated by command “rare.shared” combined the function “Shannon”, “Invsimpson”, “Shannoneven”, “Simpsoneven”. Bray-Curtis dissimilarity was calculated by “summary.shared” combined with function “Braycurtis”. OTU present-absent matrix was produced by using the command “count_table”, and then exported to XLSTAT-Ecology (Addinsoft, France) for following analyses. Principal Coordinate Analysis (PCoA),

Canonical Correspondence Analysis (Kohler, Kuo et al. 2015), Hierarchical Clustering Analysis (HCA) and Pearson correlation test were performed in XLSTAT-Ecology.

2.3 Results

2.3.1 AMF molecular diversity and identity

In total, 2592 clones were analyzed and provided 1041 clean sequences (Table 1) that were assigned to 36 operational taxonomic units (OTUs) using the 97% level of sequence similarity. Out of 36 OTUs, 27 OTUs were singletons or doubletons (they were not used for further analyses) and 9 OTUs were represented by more than two sequences and were used for in-depth analyses. However, no AMF sequences were detected in the root samples of *Persicaria maculosa*, although we retrieved some AMF sequences in its rhizospheric soil samples. Rarefaction curves reached saturation for most of the samples, indicating that our sampling effort was appropriate and sufficient to represent the AMF communities (Figure 1) except for one rhizospheric sample of *P. maculosa* in the LC site, in which only 12 sequences were obtained (Table 1). BLAST based identification and number of OTUs with shannon/simpson indexes of the AMF communities are shown in Tables 1 and S3. Generally, the values of Shannon index were between 0 and 1, which is quite low compared with the diversity showed in other studies, similar to the inverse simpson index values. However the diversity of the AMF communities associated with *P. tremuloides* and *P. maculosa* were slightly higher than the diversity of the AMF community associated with *E. elliptica,* and the variation of the AMF community associated with *E. elliptica* was lower than the variation of the AMF communities associated with *P. tremuloides* and *P. maculosa.*

A bayesian phylogenetic analysis was performed (Figure 2) to verify the identification of 9 OTUs, which clustered into five families (*Glomeraceae, Claroideoglomeraceae, Acaulosporacea, Diversisporaceae* and *Paraglomeraceae*) and six genera (*Rhizophagus, Claroideoglomus, Acaulospora, Diversispora, Paraglomus* and *Funneliformis*). Family *Glomeraceae* was dominant compared to the other families and it was represented by two genera (*Rhizophagus* and *Funneliformis*) and three virtual taxa (VTX00067, VTX00113, and VTX00114), while *Acaulosporaceae* and *Paraglomeraceae* were only represented by one OTU each. *Rhizophagus irregularis* (VTX00114) was the most dominant species covering 70.6 % of total sequences,

while the second dominant species was *Claroideoglomus* sp. (VTX00193), that represented 13.4% of the total sequences, the remaining OTUs being represented by less than 10% of the total sequences.

Interestingly, *R. irregularis* (VTX00114) was dominant in root samples of *E. elliptica*. More than 90% of the sequences assigned to *R. irregularis* (VTX00114) regardless of contamination concentrations. *R. irregularis* was also frequently found in rhizospheric soil samples with percentage of 91.7% (LC), 78.7% (MC), and 98.1% (HC) (Figure 3). *R. irregularis* (VTX00114) was still dominant in root samples from LC and HC site of *P. tremuloides* with a percentage higher than 85%, but only represented 16.7% in root samples of the MC site, where *Acaulospora* sp. (VTX00028) represented 79.2% of the total sequences. In the rhizopheric soil samples, the percentage of *R. irregularis* (VTX00114) varied greatly among samples being 52.8% in LC, 0% in MC, and 16.7% in HC sites. Meanwhile, *Claroideoglomus* sp. (VTX00193) was dominant in the rhizospheric soil samples of *P. tremuloides* with a percentage of 45.8% in LC, 70.9% in MC, and 59.1% in HC sites. For the plant species *P. maculosa*, *R. irregularis* (VTX00114) was dominant in rhizopheric soil samples from LC (91.7%) and MC (100%) sites. However, for the HC site, *R. irregularis* (VTX00114) only represented 31.3% of the sequences, while *Diversispora eburnea* (VTX00060) formed 68.8% of the sequences.

2.3.2 AMF community structure

A Principal Coordinate Analysis (PCoA) discriminated the AMF communities associated with *E. elliptica,* showing a remarkably low level of variation between root and soil samples (Figure 4). This was supported by a Hierarchical Clustering Analysis (HCA), which clustered all the AMF communities associated with *E. elliptica* in one group together with the root samples of *P. tremuloides* from the LC and HC sites (Figure S4). On the other hand, in *P. tremuloides*, AMF communities showed clear differences between root and soil samples with the separation of both F1 (35.9%) and F2 (21.1%) axes, which was also supported in the HCA with clearly separated clustering of soil samples against root samples of *P. tremuloides*. AMF communities associated with the rhizosphere of *P. maculosa* were largely distict from those associated with by both *P. tremuloides* and *E. elliptica*, that were clearly separated by the PcoA with a distinct clustering also visible in the HCA.

The influence of different contamination levels on AMF community structure was found in the samples from the host plant *E. elliptica.* The AMF community from the LC site was separated from those from MC and HC sites on the second principal coordinate F2 explaining a variance of 21.1%. A Hierarchical Clustering Analysis also supported this observation by showing clustering of MC and HC sites (with >80% similarity) separated from the LC site. For roots samples of *P. tremuloides*, the AMF community from MC was clearly separated with those from LC and HC sites, which corresponded with the results of the Hierarchical Clustering Analysis.

A Canonical Correspondence Analysis was performed with 1000 Monte-Carlo permutations to analyze the relationship of individual AMF OTUs with three environmental factors: host plants species (*P. tremuloides, E. elliptica and P. maculosa*), contamination levels (low contaminated sites, moderate contaminated sites and high contaminated sites) and biotopes (roots and soil) (Figure 5). Some AMF species showed certain association with host plants. OTU1 (*R. irregularis,* VTX00114) and OTU7 (*Paraglomus* sp., VTX00350) showed some association with *E. elliptica* roots, while OTU3 (*Acaulospora* sp., VTX00276) was mostly associated with *P. tremuloides* roots. OTU2 (*Claroideoglomus* sp., VTX00193), OTU6 (*Claroideoglomus* sp., VTX00276), OTU8 (*Funneliformis mosseae*, VTX00067), and OTU9 (*Diversispora celeta*, VTX00060) were likely associated with the rhizospheric soil of *P. tremuloides*. OTU4 (*Rhizophagus* sp., VTX00113) and OTU5 (*Diversispora eburnea*, VTX00060) were mostly associated with *P. maculosa.* In addition, we observed the correlation of some OTUs with different contaminated sites. OTU1(*R. irregularis,* VTX00114) and OTU7 (*Paraglomus* sp., VTX00350) were frequently present in the low contaminated site, while OTU3 (*Acaulospora* sp., VTX00276) and OTU9 (*Diversispora celeta*, VTX00060) were mostly found in the moderate contaminated site. In addition, OTU5 (*Diversispora eburnea*, VTX00060), OTU6 (*Claroideoglomus* sp., VTX00276) and OTU8 (*Funneliformis mosseae*, VTX00067) likely appeared in the highly contaminated sites. Moreover, OTU1 (*R. irregularis,* VTX00114), OTU4 (*Rhizophagus* sp., VTX00113) and OTU7 (*Paraglomus* sp., VTX00350) were mostly found in roots samples and OTU2 (*Claroideoglomus* sp., VTX00193), OTU5 (*Diversispora eburnea*, VTX00060), OTU6 (*Claroideoglomus* sp., VTX00276) and OTU8 (*Funneliformis mosseae*, VTX00067) were frequently presented in soil samples.

To specifically show the impact of contamination levels on the abundance of AMF species, a Pearson correlation analysis was performed on the major AMF OTUs of roots and soil samples from both *E. elliptica* and *P. tremuloides* across the three different contaminated sites.

We found that *Eleocharis elliptica* root samples were largely dominated by *R. irregularis* (VTX00114) regardless of the site with minor shifting of the relative abundance in LC, where it was partly replaced by *Paraglomus* sp. (VTX00350) (Figure 6a). However, there was no significant correlation between hydrocarbon pollutants and these two OTUs (Table S5). In the rhizospheric soil of *E. elliptica*, the relative abundance of *R. irregularis* (VTX00114) was partly replaced by *Rhizophagus* sp. (VTX00113). Meanwhile, no significant correlation between hydrocarbon contamination and these two OTUs was observed. *Claroideoglomus* sp. (VTX00193) was only found in LC, and was not detected in MC and HC (Figure 3; 6b). This pattern seemed to be related with its negative correlation with hydrocarbon contamination ($P < 0.05$).

A similar pattern was observed in *P. tremuloides* where shifting of relative abundances along the contaminated sites also occurred (Figure 6c and 6d). In the root of *P. tremuloides*, the relative abundance of *Acaulospora* sp. (VTX00276) showed different pattern of shifting with those of *R. irregularis* and *Rhizophagus* sp. Change of relative abundance of two *Rhizophagus* OTUs seemed to be affected by their negative correlation with pollutants (not significant, but notable: *R. irregularis* $P = 0.083$ with acenaphthene, *Rhizophagus* sp. $P = 0.07$ with acenaphthene, $P = 0.093$ with fluorene) (Table S7). Interestingly, the two *Rhizophagus* OTUs showed totally different correlation patterns in association with *E. elliptica* and *P. tremuloides*.

In the soil sample of *P. tremuloides*, *R. irregularis* was dominant in LC, but showed low relative abundance in HC and was not detected in MC. This is likely to be related with its negative correlation with pollutant ($P < 0.05$)(Table S8).

2.4 Discussion

We report AMF community structure changes with specific patterns in three sites of different petroleum hydrocarbon contamination concentrations. The change of AMF community structures is not only affected by petroleum hydrocarbon concentration, but also highly correlated with host plant species and biotopes. Relative abundance changes and PCoA confirmed the influences of biotopes and hydrocarbon contamination on AMF community structure, which showed different shifts among host plant species. For example, AMF communities associated with *E. elliptica* were

significantly affected by the concentration of petroleum hydrocarbons compared to biotopes, while communities associated with *Populus tremuloides* showed a clear difference between biotopes regardless of petroleum hydrocarbons concentration. It is well documented that host plant species can strongly influenced their AMF community structures (Torrecillas, Alguacil et al. 2012, Yang, Zang et al. 2012, Yang, Zang et al. 2012, Guo and Gong 2014). However, the dependency of AMF community structure on host plant species under petroleum hydrocarbon contamination remained unclear.

Previous studies reported that host plant species could favor some AMF species to become dominant in their roots under certain environmental conditions (Eom, Hartnett et al. 2000, Su, Sun et al. 2011). In contrast, earlier studies suggested that there is no absolute specificity between AMF taxa and their host plants (Magrou 1936, Stahl 1949, Gerdemann 1955); the authors argued that each AMF species could colonize the roots of more than one host plant at the same time, while each mycorrhizal plant could be colonized by more than one AMF taxon. However, evidences have been reported in the support of a narrowed specificity of effective symbiotic partners due to the dialog response of both AMF and plants, which could exert selection pressure (Smith and Read 2008). Govannetti (1985) found that an intimate AMF plant specificity existed between three legume species, *Medicago sativa*, *Hedysarum coronarium* and *Onobrychis viciaefolia,* and four *Glomus* species when grown in two soils with different phosphorus (P) availability.

In our study, soil samples of *P. tremuloides* showed that *Claroideoglomus* sp. (VTX00193) was the dominant OTU, while those of *E. elliptica* have *R. irregularis* as dominant OTU. This difference was observed only in the rhizosphere soil samples of *E. elliptica* and *P. tremuloides*. However, all of the root samples regardless of host plant species (except one root sample of *P. tremuloides* from MC which showed *Aculospora* sp. (VTX00028) as dominant species) did not show any difference in dominant AMF OTUs. Instead, they shared *R. irregularis* as a common dominant AMF OTU. Nevertheless, these three different host plant species showed clear differences in the composition of AMF community structure.

We found that AMF OTUs were highly correlated with three environmental factors (contamination concentration, host plant identity, biotope), which is in line with Velazquez, Cabello et al. (2013) who reported that AMF occurrence under certain environmental condition varied significantly between species. In our study, *Paraglomus* sp. had a strong correlation with

low contamination, while *F. mosseae* showed the opposite pattern, and was highly correlated with high contamination. Many reports suggested that *F. mosseae* had high tolerance in extreme environments (Xu *et al*., 2008, Barti *et al*., 2013, Vivas *et al*., 2003) and it was also frequently found in trace metals contaminated sites (Hassan et al. 2011).

Interestingly, the highly dominant species found in most of our samples, *R. irregularis*, showed a strong negative correlation with petroleum pollutants in *P. tremuloides* soil samples. *R. irregularis* is one of the most common AMF species which is frequently found in diverse ecosystems. It was also reported as dominant AMF species in various contaminated sites, such as trace metals and petroleum hydrocarbons contaminated site (Hassan et al. 2011, Hassan, Bell et al. 2014, de la Providencia, Stefani et al. 2015), but unlike the results showed in Hassan, Bell et al. (2014), who found that the abundance of *R. irregularis* increased in highly contaminated sites, our results suggest that *R. irregularis* is significantly affected by the hydrocarbon pollutants and its abundance actually decreased in the highly contaminated site when it was associated with the rhizosphere of *P. tremuloides*. Thus, the difference in abundance of *R. irregularis* between our study and previous studies could be related with complex relationships between AMF OTUs and contamination concentrations, different sampling time, and the different host plant species we chose for study.

We found that there was a higher diversity of AMF species associated with *P. tremuloides* compared with *E. elliptica*, which suggested this plant species may have more advantage for future application of phytoremediation in petroleum hydrocarbon contaminated sites.

In addition, Cano and Bago (2005) cultured *Glomus intraradices* (synonym: *R. irregularis*), *Glomus proliferum*, and *Gigaspora margarita* in an *in vitro* compartmented petri dish system and showed intrinsic differences among AMF taxa on root colonization. This rises the question to whether AMF species of the same phylogenetic clade have similar patterns of distribution against contamination concentrations and biotopes. We found several OTUs belonging to the order *Diversisporales* (*Acaulospora* sp., *Diversispora eburnea*, and *Diversispora celeta*) and the family *Claroideoglomeraceae* (*Claroideoglomus* sp. (VTX00193), *Claroideoglomus* sp. (VTX00276)). The CCA revealed that *Diversisporales* did not show unified patterns of distribution with any of our three environmental factors: host plant, contamination level and biotope. However, the OTUs of *Diversisporales* showed the tendency to cluster between MC and HC sites, apart from those of LC. OTUs of *Claroideoglomeraceae* family showed correspondence with host plant species and

biotopes, especially in the rhizospheric soil of *P. tremuloides*. For the hydrocarbon contamination, *Claroideoglomus* sp. (VTX00276) and *Claroideoglomus* sp. (VTX00193) both showed some correspondances with HC and MC site.

We report that some AMF OTUs showed unique preference with host plant identity, biotopes, sites, and hydrocarbon pollutant concentrations. In addition, different OTUs belonging to the same family showed both similarities and differences in the canonical analyses with these environmental factors.

A previous study showed correlations between AMF species in single roots system (Alkan, Gadkar et al. 2006). In our results, Pearson correlation analysis along with relative abundance showed a clear shift of negative correlations between AMF OTUs and pollutants. However, no significant correlation between AMF OTUs and pollutants was detected in root samples of both *E. elliptica* and *P. tremuloides*. This could be explained by the fact that AMF inside of roots are not directly in contact with pollutants, in other word, the roots may serve a protecting barrier against pollutant toxicity. We observed negative correlations between *Claroideoglomus* sp. (*VTX00193*) and petroleum pollutants in *E. elliptica* soil samples, and also negative correlations between *R. irregularis* and petroleum pollutants in *P. tremuloides* soil samples. This could be due the stress of AMF communities in contact with pollutants in the soil.

In summary, we showed that plant identity, biotopes and petroleum contamination concentrations affected AMF OTUs abundance and influenced their community structure. We also report that interactions between these environmental factors and AMF OTUs. Overall, *R. irregularis* was found to be the dominant OTU in the three sites, but it showed a negative correlation with petroleum hydrocarbon contamination in *P. tremuloides* soil samples as per Pearson correlation analysis. On the contrary, *Diversisporales* and *Claroideoglomuraceae* showed certain correspondence with hydrocarbon contamination (MC and HC) in CCA. Further investigation using *Diversisporales* and *Claroideoglomeraceae* taxa is needed to better understand their interactions and community structures in extreme environments.

2.5 Acknowledgements

This work was supported by The Natural Sciences and Engineering Research Council of Canada (NSERC) which is greatly acknowledged. We thank Stéphane Daigle for assistance in statistics. We thank Karen Fisher-Favret for commenting on and editing the manuscript.

2.5 References

Alkan N, Gadkar V, Yarden O & Kapulnik Y (2006) Analysis of quantitative interactions between two species of arbuscular mycorrhizal fungi, Glomus mosseae and G. intraradices, by real-time PCR. *Appl Environ Microbiol* **72**: 4192-4199.

Azaizeh HA, Marschner H, Romheld V & Wittenmayer L (1995) Effects of a Vesicular-Arbuscular Mycorrhizal Fungus and Other Soil-Microorganisms on Growth, Mineral Nutrient Acquisition and Root Exudation of Soil-Grown Maize Plants. *Mycorrhiza* **5**: 321-327.

Barea JM, Pozo MJ, Azcon R & Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. *Journal of experimental botany* **56**: 1761-1778.

Bell TH, Hassan SE, Lauron-Moreau A, Al-Otaibi F, Hijri M, Yergeau E & St-Arnaud M (2014) Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *Isme J* **8**: 331-343.

Brundrett M (1991) Mycorrhizas in Natural Ecosystems. *Adv Ecol Res* **21**: 171-313.

Cabello MN (1999) Effectiveness of indigenous arbuscular mycorrhizal fungi (AMF) isolated from hydrocarbon polluted soils. *J Basic Microb* **39**: 89-95.

Cano C & Bago A (2005) Competition and substrate colonization strategies of three polyxenically grown arbuscular mycorrhizal fungi. *Mycologia* **97**: 1201-1214.

Darriba D, Taboada GL, Doallo R & Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* **9**: 772-772.

Davies FT, Potter JR & Linderman RG (1993) Drought Resistance of Mycorrhizal Pepper Plants Independent of Leaf P-Concentration - Response in Gas-Exchange and Water Relations. *Physiologia Plantarum* **87**: 45-53.

Davies FT, Puryear JD, Newton RJ, Egilla JN & Grossi JAS (2002) Mycorrhizal fungi increase chromium uptake by sunflower plants: Influence on tissue mineral concentration, growth, and gas exchange. *J Plant Nutr* **25**: 2389-2407.

de la Providencia IE, Stefani FOP, Labridy M, St-Arnaud M & Hijri M (2015) Arbuscular mycorrhizal fungal diversity associated with Eleocharis obtusa and Panicum capillare growing in an extreme petroleum hydrocarbon-polluted sedimentation basin. *FEMS microbiology letters* **362**.

Desjardins D, Nissim WG, Pitre FE, Naud A & Labrecque M (2014) Distribution patterns of spontaneous vegetation and pollution at a former decantation basin in southern Quebec, Canada. *Ecol Eng* **64**: 385-390.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**: 1792-1797.

Eom AH, Hartnett DC & Wilson GWT (2000) Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia* **122**: 435-444.

Estrada-Luna AA, Davies FT & Egilla JN (2000) Mycorrhizal fungi enhancement of growth and gas exchange of micropropagated guava plantlets (Psidium guajava L.) during ex vitro acclimatization and plant establishment. *Mycorrhiza* **10**: 1-8.

Gerdemann J (1955) Relation of large soil-born spore to phycomycetous mycorrhizal infections. *Mycologia* **47**: 619-632.

Govannetti M (1985) Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. *T Brit Mycol Soc* **84**: 679-684.

Guo XH & Gong J (2014) Differential effects of abiotic factors and host plant traits on diversity and community composition of root-colonizing arbuscular mycorrhizal fungi in a salt-stressed ecosystem. *Mycorrhiza* **24**: 79-94.
Hassan SE, Hijri M & St-Arnaud M (2013) Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil. *New Biotechnol* **30**: 780-787.
Hassan SED, Boon E, St-Arnaud M & Hijri M (2011) Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. *Molecular ecology* **20**: 3469-3483.
Hassan SED, Bell TH, Stefani FOP, Denis D, Hijri M & St-Arnaud M (2014) Contrasting the Community Structure of Arbuscular Mycorrhizal Fungi from Hydrocarbon-Contaminated and Uncontaminated Soils following Willow (Salix spp. L.) Planting. *PloS one* **9**.
Hijri M (2016) Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. *Mycorrhiza* **26**: 209-214.
Jeffries P, Gianinazzi S, Perotto S, Turnau K & Barea J-M (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils* **37**: 1-16.
Johansson JF, Paul LR & Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS microbiology ecology* **48**: 1-13.
Kohler, A., A. Kuo, L. G. Nagy, E. Morin, K. W. Barry, F. Buscot, B. Canback, C. Choi, N. Cichocki, A. Clum, J. Colpaert, A. Copeland, M. D. Costa, J. Dore, D. Floudas, G. Gay, M. Girlanda, B. Henrissat, S. Herrmann, J. Hess, N. Hogberg, T. Johansson, H.-R. Khouja, K. LaButti, U. Lahrmann, A. Levasseur, E. A. Lindquist, A. Lipzen, R. Marmeisse, E. Martino, C. Murat, C. Y. Ngan, U. Nehls, J. M. Plett, A. Pringle, R. A. Ohm, S. Perotto, M. Peter, R. Riley, F. Rineau, J. Ruytinx, A. Salamov, F. Shah, H. Sun, M. Tarkka, A. Tritt, C. Veneault-Fourrey, A. Zuccaro, C. Mycorrhizal Genomics Initiative, A. Tunlid, I. V. Grigoriev, D. S. Hibbett and F. Martin (2015). "Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists." *Nat Genet* **47**(4): 410-415.
Kruger M, Kruger C, Walker C, Stockinger H & Schussler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytologist* **193**: 970-984.
Lee J, Lee S & Young JP (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS microbiology ecology* **65**: 339-349.
Magrou M (1936) *Culture et inoculation du champignon de mycorhizes a arbuscules et a vesicules*.
Opik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier U & Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223-241.
Pawlowska TE, Chaney RL, Chin M & Charvat I (2000) Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. *Appl Environ Microbiol* **66**: 2526-2530.
Rivera-Becerril F, Calantzis C, Turnau K, Caussanel J-P, Belimov AA, Gianinazzi S, Strasser RJ & Gianinazzi-Pearson V (2002) Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three Pisum sativum L. genotypes. *Journal of experimental botany* **53**: 1177-1185.
Roy-Bolduc A & Hijri M (2011) The Use of Mycorrhizae to Enhance Phosphorus Uptake: A Way Out the Phosphorus Crisis. *J Biofertil Biopestici* **2**: 104.

Schloss PD, Westcott SL, Ryabin T, *et al.* (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl Environ Microbiol* **75**: 7537-7541.
Smith & Read (2008) Mycorrhizal Symbiosis. p.^pp. 800. *Academic Press*.
Smith SE & Barker SJ (2002) Plant phosphate transporter genes help harness the nutritional benefits of arbuscular, mycorrhizal symbiosis. *Trends in plant science* **7**: 189-190.
Stahl JD (1949) Die mycorrhiza de lebermoose mit besonderer beruchsichtigung der thallosen formen. *Planta* **37**: 103-148.
St-Arnaud M & Vujanovic V (2007) Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. *Mycorrhizae in Crop Production*,(Hamel C & Plenchette C eds.), pp. 67-122. Haworth Food & Agricultural Products Press, Binghamton NY.
Su YY, Sun X & Guo LD (2011) Seasonality and Host Preference of Arbuscular Mycorrhizal Fungi of Five Plant Species in the Inner Mongolia Steppe, China. *Braz J Microbiol* **42**: 57-65.
Torrecillas E, Alguacil MM & Roldan A (2012) Host Preferences of Arbuscular Mycorrhizal Fungi Colonizing Annual Herbaceous Plant Species in Semiarid Mediterranean Prairies. *Appl Environ Microbiol* **78**: 6180-6186.
Velazquez MS, Cabello MN & Barrera M (2013) Composition and structure of arbuscular-mycorrhizal communities in El Palmar National Park, Argentina. *Mycologia* **105**: 509-520.
Yang, H., Y. Zang, Y. Yuan, J. Tang and X. Chen (2012). "Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: evidence from ITS rDNA sequence metadata." *BMC Evol Biol* **12**: 50.
Yang, Y. R., X. Z. Han, Y. Liang, A. Ghosh, J. Chen and M. Tang (2015). "The Combined Effects of Arbuscular Mycorrhizal Fungi (AMF) and Lead (Pb) Stress on Pb Accumulation, Plant Growth Parameters, Photosynthesis, and Antioxidant Enzymes in Robinia pseudoacacia L." *Plos One* **10**(12).

Table 1. Summary of sampling information. Shannon index and Simpson index were calculated on each group to estimate the alpha-diversity. Communities from low, moderate and high contaminated sites were presented as LC, MC and HC, while communities associated with soil samples and roots samples were presented as R and S.

Group	num seqs	num OTUs	shannon	invsimpson	shannoneven	simpsoneven
*E. elliptica*_HC_R	53	2	0.0936	1.039216	0.135036	0.519608
*E. elliptica*_HC_S	52	2	0.09503	1.04	0.137099	0.52
*E. elliptica*_LC_R	124	3	0.275497	1.140592	0.250768	0.380197
*E. elliptica*_LC_S	121	3	0.326537	1.183567	0.297227	0.394522
*E. elliptica*_MC_R	57	2	0.08832	1.036364	0.127419	0.518182
*E. elliptica*_MC_S	61	2	0.518054	1.517413	0.747394	0.758706
*P. maculosa*_HC_S	32	2	0.621086	1.797101	0.896038	0.898551
*P. maculosa*_LC_S	12	2	0.286836	1.2	0.413817	0.6
*P. maculosa*_MC_S	21	1	0	1	1	1
*P. tremuloides*_HC_R	112	4	0.489046	1.297975	0.352772	0.324494
*P. tremuloides*_HC_S	66	4	1.103597	2.491289	0.796077	0.622822
*P. tremuloides*_LC_R	107	2	0.241629	1.140817	0.348597	0.570408
*P. tremuloides*_LC_S	72	3	0.754263	2.076361	0.68656	0.69212
*P. tremuloides*_MC_R	72	4	0.642512	1.536981	0.463474	0.384245
*P. tremuloides*_MC_S	79	4	0.901381	1.891344	0.650209	0.472836

Note: the number of the AMF sequences abtained from each group (num seqs); the number of the OTUs abtained from each group (num OTUs); shannon diversity index (shannon); invers simpson diversity index (invsimpson); shannon evenness index (shannoneven); simpson evenness index (simpsoneven)

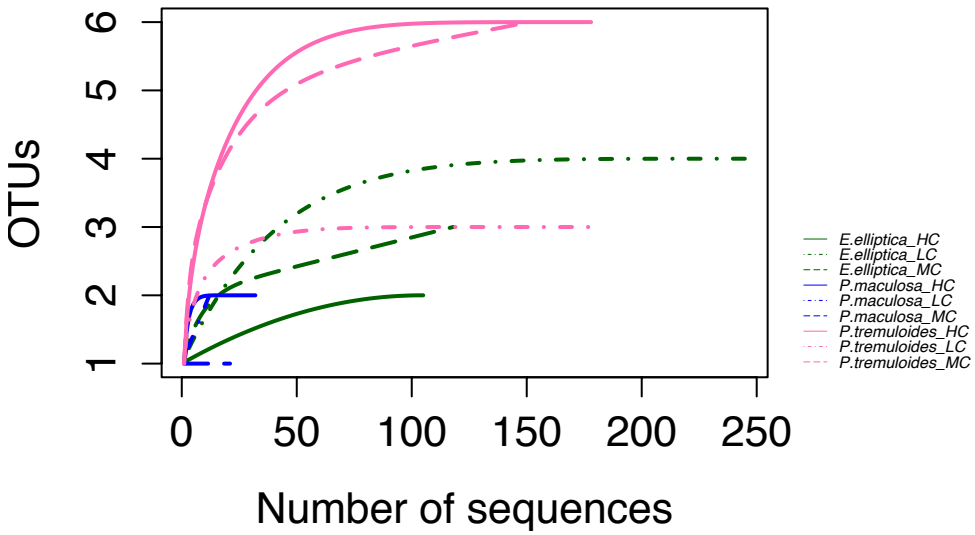
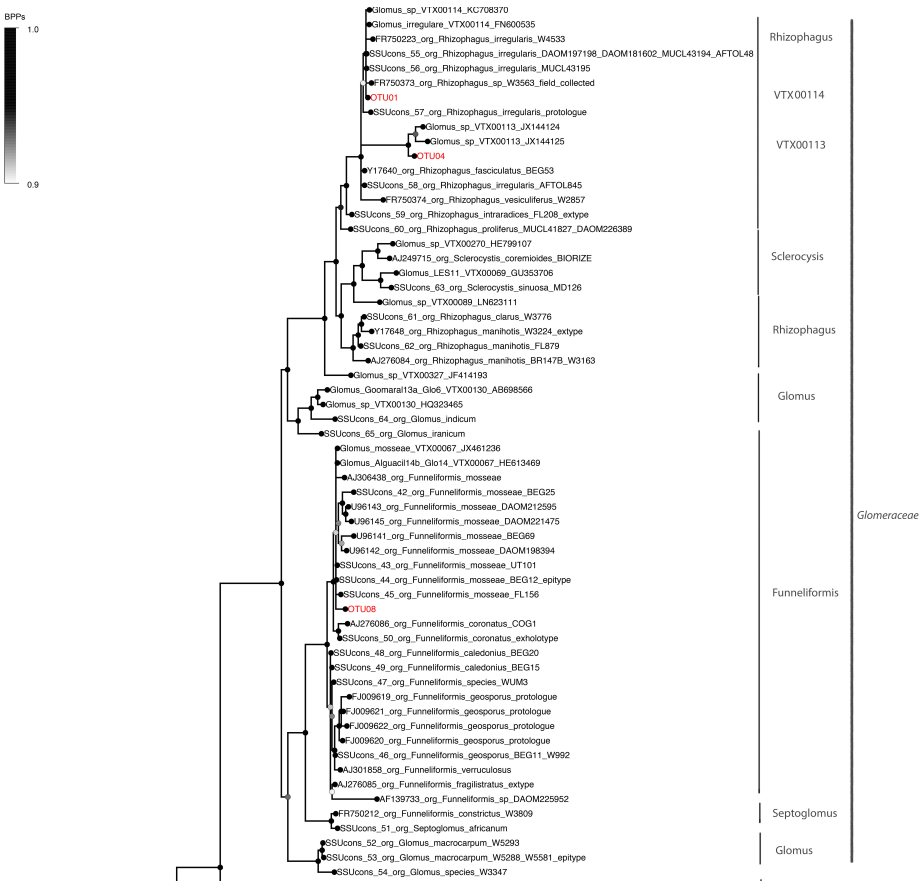


Figure 1. Rarefaction curves showing the saturation of OTUs richness of *Glomeromycota* associated with *P. tremuloides*, *E. elliptica* and *P. maculosa* from low, moderate and high contaminated sites. Rarefaction analysis was based on taxonomic assignment at 97% of sequence similarity.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0.9
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SSUcons_55_org_Rhizophagus_irregularis_DAOM197198_DAOM181602_MUCL43194_AFTOL48
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FR750373_org_Rhizophagus_sp_W3563_field_collected
OTU01
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Glomus_sp_VTX00113_JX144124
Glomus_sp_VTX00113_JX144125
OTU04
Y17640_org_Rhizophagus_fasciculatus_BEG53
SSUcons_58_org_Rhizophagus_irregularis_AFTOL845
FR750374_org_Rhizophagus_vesiculiferus_W2857
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U96142_org_Funneliformis_mosseae_DAOM198394
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OTU08
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FJ009621_org_Funneliformis_geosporus_protologue
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AJ276085_org_Funneliformis_fragilistratus_extype
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FR750212_org_Funneliformis_constrictus_W3809
SSUcons_51_org_Septoglomus_africanum
SSUcons_52_org_Glomus_macrocarpum_W5293
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SSUcons_54_org_Glomus_species_W3347
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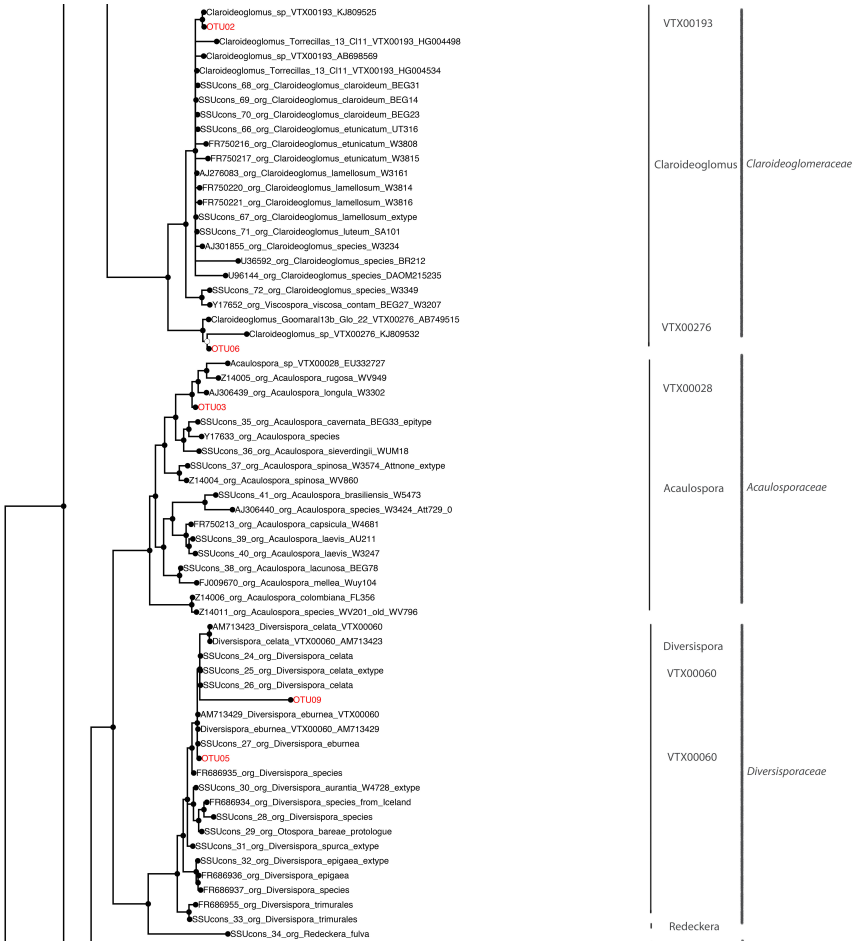
VTX00114
VTX00113
Sclerocysis
Rhizophagus
Glomus
Funneliformis
Septoglomus
Glomus
Glomeraceae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U36592_org_Claroideoglomus_species_BR212
U96144_org_Claroideoglomus_species_DAOM215235
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Claroideoglomus
VTX00276
Claroideoglomeraceae
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Acaulospora
Acaulosporaceae
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Redeckera

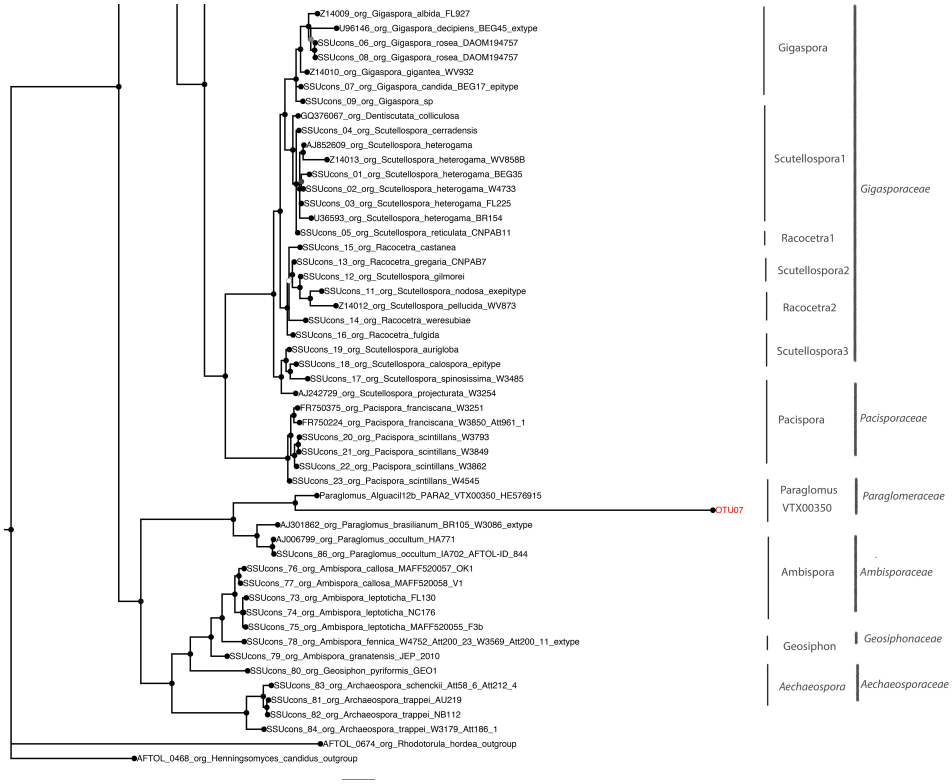

Figure 2. Bayesian phylogenetic tree based on nuclear small subunit (SSU) rDNA consensus sequences showing the distribution of the 9 OTUs recorded in contaminated sites (red labels) among the Glomeromycota phylogenetic tree. Sequence dataset was analyzed with the SSU sequences (black labels) from Krüger et al. (2012) and the closest match recovered from MaarjAM database. Circles on nodes indicate Bayesian posterior probabilities ranging from 0.9 (white) to 1 (black). The scale represents the branch length corresponding to expected substitutions per site.

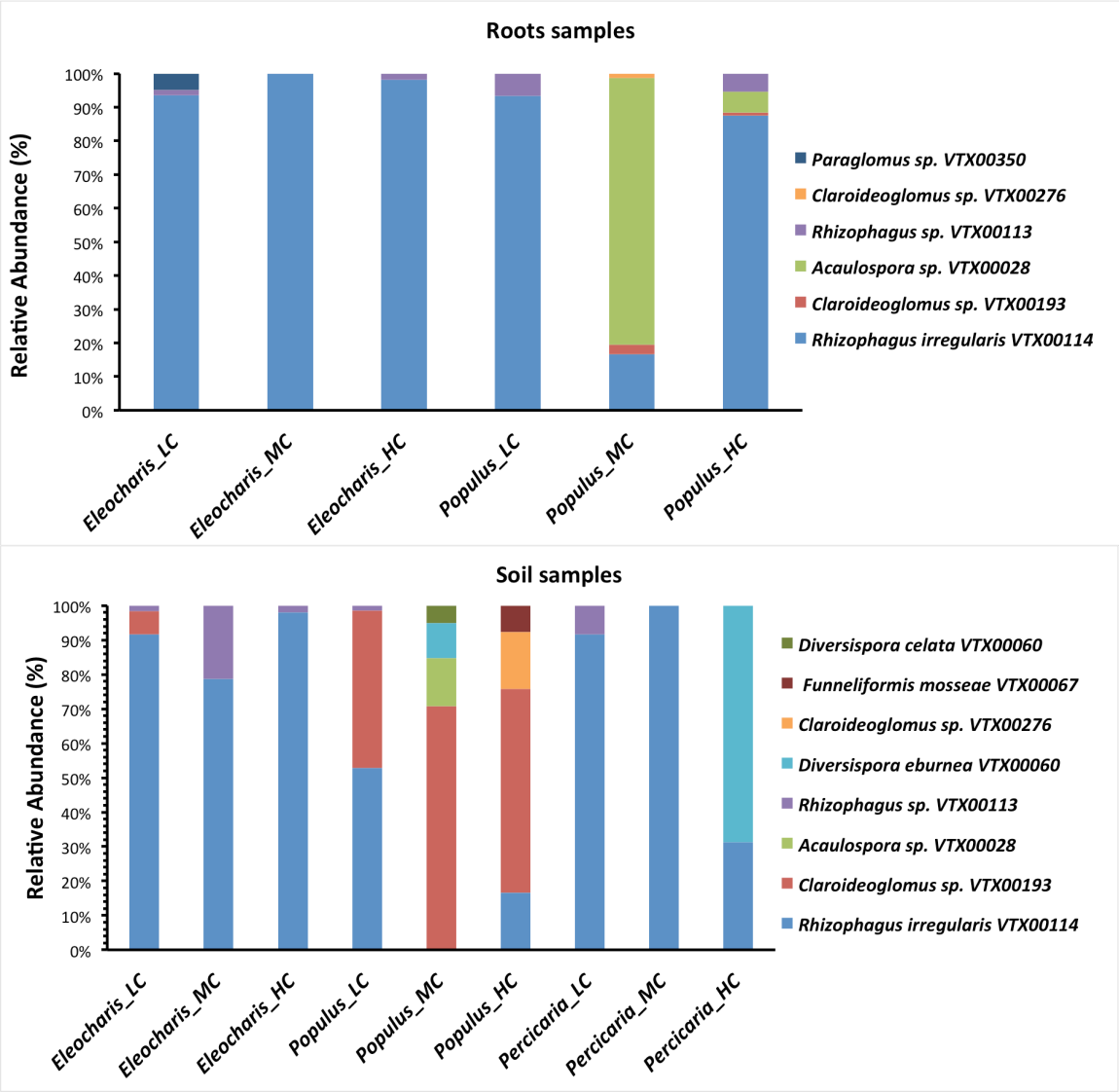

Figure 3. a. Relative abundance of 6 OTUs of *Glomeromycota* within roots samples from *P. tremuloides* in low, moderate and high contaminated sites (*Populus*_LC, *Populus*_MC and *Populus*_HC) **and from *E. elliptica* in low, moderate and high contaminated sites** (*Eleocharis*_LC, *Eleocharis*_MC and *Eleocharis*_HC). **b. Relative abundance of 8 OTUs of *Glomeromycota* within rhizospheric soil samples from *P. tremuloides* in low, moderate and high contaminated sites** (*Populus*_LC, *Populus*_MC and *Populus*_HC) **and from *E. elliptica* in low, moderate and high contaminated sites** (*Eleocharis*_LC, *Eleocharis*_MC and *Eleocharis*_HC) **and from *P. maculosa* in low, moderate and high contaminated sites** (*Persicaria*_LC, *Persicaria*_MC and *Persicaria*_HC).

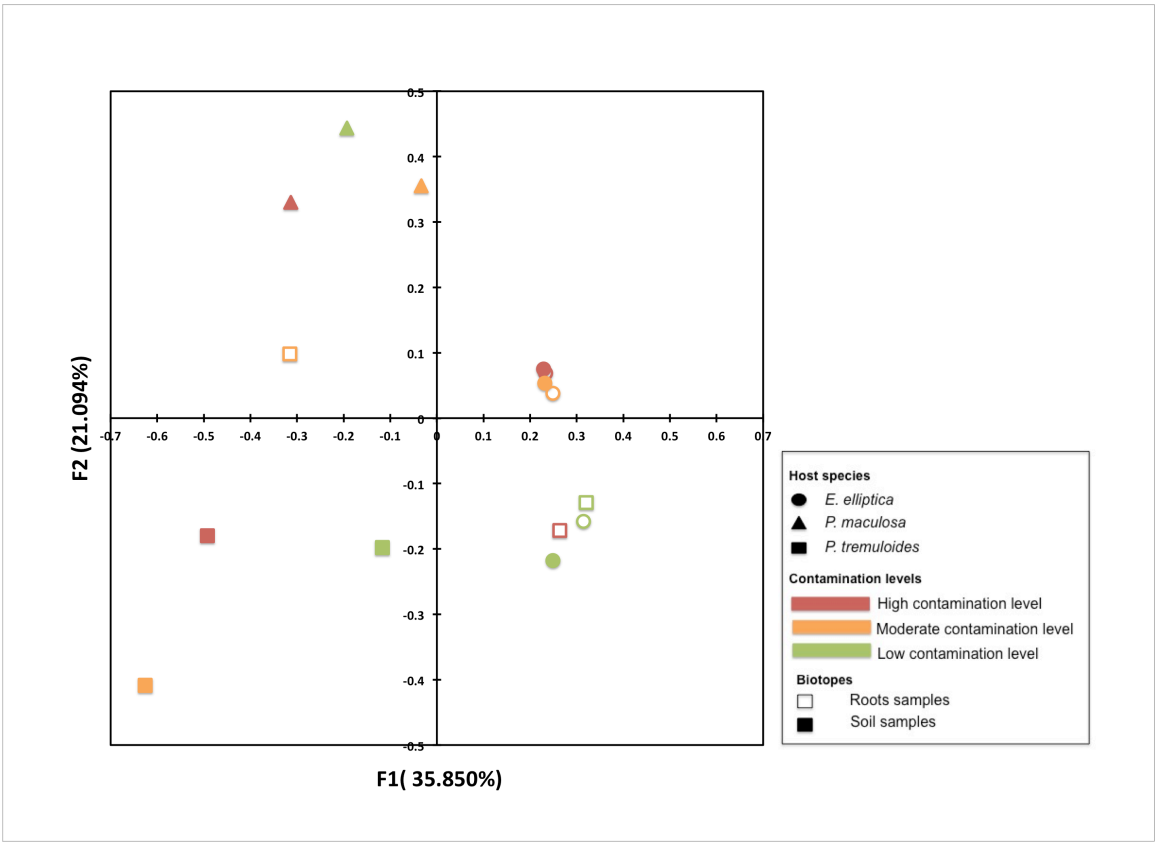

Figure 4. Principal coordinate analysis (PCoA) performed on AMF communities in rhizospere soil (solid**) and roots (hollow) associated with *P. tremuloides* (square), *E. elliptica* (round) and *P. maculosa* (triangle) from low contaminated sites (green colour), moderate contaminated site (yellow colour) and high contaminated site (red colour) based on Bray-Curtis dissimilarity. Axis 1 explains 35.850% variation of the community composition, while axis 2 explains 21.094% variation.**

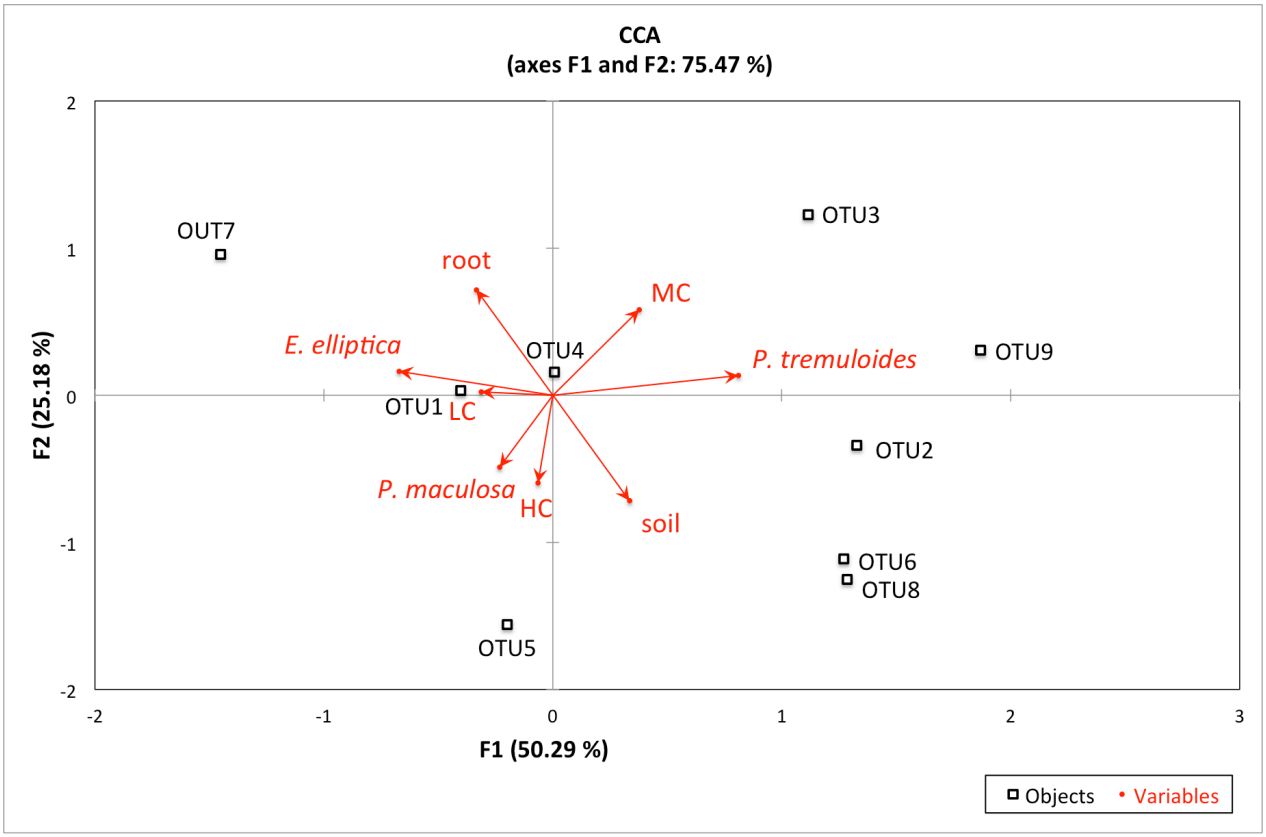

Figure 5. Canonical Correspondence Analysis showing the relationship between AMF OTU assemblages with three environmental factors: host plants species (*P. tremuloides*, *E. elliptica* and *P. maculosa*), contamination levels (LC as low contaminated sites, MC as moderate contaminated sites and HC as high contaminated sites) and biotopes(roots and soil). . A 1000 replicates of Monte-Carlo permutation test was applied.

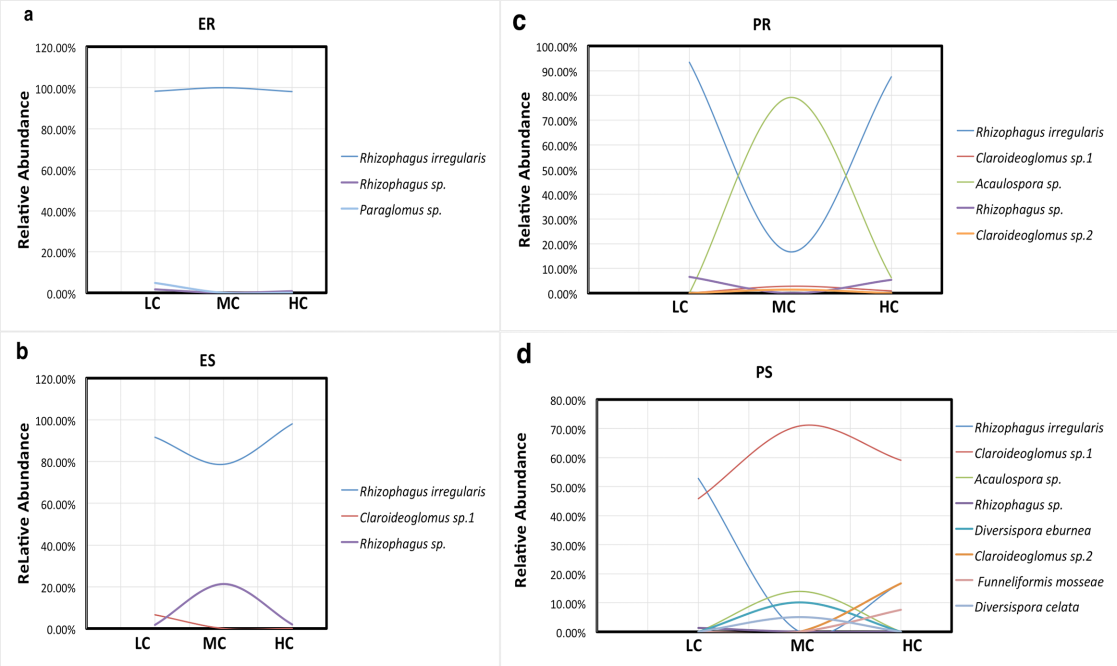

Figure 6. Changes of abundance of major AMF OTUs across the three different contamination sites (LC as low contaminated site, MC as moderate contaminated site and HC and high contaminated site). a. Abundance change of major AMF OTUs associated with *E. elliptica* roots samples (ER); b. Abundance change of major AMF OTUs associated with *E. elliptica* soil samples (ES); c. Abundance change of major AMF OTUs associated with *P. tremuloides* roots samples (PR); d. Abundance change of major AMF OTUs associated with *P. tremuloides* soil samples (PS).

Supporting material

Figure S1. Aerial view and location of the three basins and sampling sites in each basin (red label).

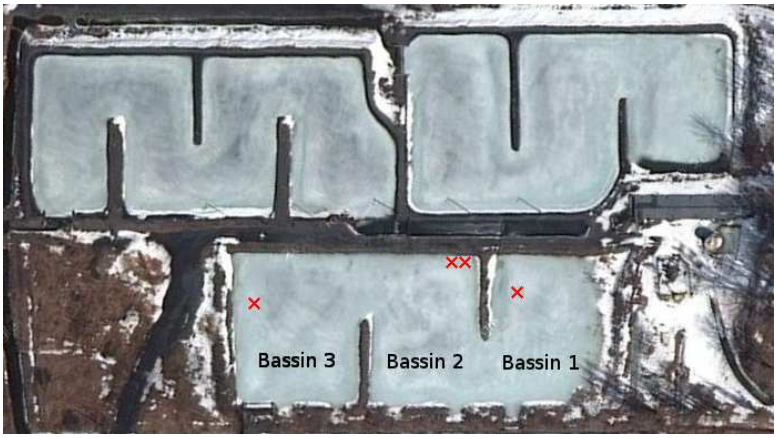

Table S2. The concentration of different petroleum hydrocarbon pollutants recorded in the three sampling sites compared with the reference values set by Quebec Government.

Polycyclic aromatic hydrocarbons	Concentration recorded in different sites ***µg/kg***			Reference values set by Quebec Government (ccme.ca)		
	LC	MC	HC	Residential	Commercial	Industrial
Phenanthrene	1	2700	4300	0.1	5	50
Acenaphrene	0.2	760	620	0.1	10	100
Anthracene	6.7	340	570	0.1	10	100
Fluorene	0.3	710	630	0.1	10	100
1-methylnaphtalene	0.1	320	300	0.1	1	10
1,3-Dimethylnaphtalene	0.1	390	580	0.1	1	10
Total Petroleum Hydrocarbons (C10-C50)	3000	41000	91000	300	700	3500

Table S3. Identification of OTUs. BLAST results (using NCBI and MarriamJ database) of the 36 OTUs were shown below. OTUs represented by 2 or less than 2 sequences (marked as red color) were not be used for future analysis.

OTUs	Identification	*Orders*	*Families*	*Genera*	Accessing code.	Similarity
OTU01	*Rhizophagus irregulare VTX00114*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	FN600536.1	100%
OTU02	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KJ809525.1	100%
OTU03	*Acaulospora sp. VTX00028*	*Diversisporale*	*Acaulosporacea*	*Acaulospora*	EU332727.1	99%
OTU04	*Rhizophagus sp. VTX00113*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	JX144124.1	99%
OTU05	*Diversispora eburnea VTX00060*	*Diversisporale*	*Diversisporaceae*	*Diversispora*	AM713429.1	100%
OTU06	*Claroideoglomus sp. VTX00276*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	AB749515.1	99%
OTU07	*Paraglomus sp. VTX00350*	*Paraglomerale*	*Paraglomeraceae*	*Paraglomus*	HE576915.1	91%
OTU08	*Funneliformis mosseae VTX00067*	*Glomerale*	*Glomeraceae*	*Funneliformis*	JX461236.1	98%
OTU09	*Diversispora celata VTX00060*	*Diversisporale*	*Diversisporaceae*	*Diversispora*	AM713423.1	96%
OTU10	*Rhizophagus sp. VTX00114*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	KC708370	98%
OTU11	*Glomeraceae sp. VTX00327*	*Glomerale*	*Glomeraceae*	-	JF414193	94%
OTU12	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KJ809525.1	95%
OTU13	*Glomeraceae sp. VTX00069*	*Glomerale*	*Glomeraceae*	-	GU353706.1	96%
OTU14	*Glomeraceae sp. VTX00130*	*Glomerale*	*Glomeraceae*	-	AB698566.1	99%
OTU15	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KJ809525.1	97%
OTU16	*Rhizophagus irregulare VTX00114*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	FN600538.1	97%
OTU17	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KJ809525.1	97%
OTU18	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KJ809525.1	95%
OTU19	*Rhizophagus sp.VTX00113*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	HG004504.1	96%
OTU20	*Rhizophagus sp.VTX00113*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	HG004476.1	97%
OTU21	*Rhizophagus irregulare VTX00114*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	JX144120.1	97%
OTU22	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	HQ258987.1	96%
OTU23	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KJ809525.1	98%
OTU24	*Rhizophagus sp.VTX00113*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	HG004521.1	99%
OTU25	*Claroideoglomus sp.VTX00057*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KC708358.1	97%
OTU26	*Rhizophagus sp.VTX00113*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	EU332711.1	96%
OTU27	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	KJ809525.1	98%
OTU28	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	HE615035.1	95%
OTU29	*Glomeraceae Glomus sp. VTX00114*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	KC708370.1	99%
OTU30	*Claroideoglomus sp. VTX00193*	*Glomerale*	*Claroideoglomeraceae*	*Claroideoglomus*	HE614983.1	97%
OTU31	*Paraglomus sp. VTX00001*	*Paraglomerale*	*Paraglomeraceae*	*Paraglomus*	KF386326.1	89%
OTU32	*Rhizophagus irregulare VTX00114*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	KC708370.1	99%
OTU33	*Glomeraceae sp.VTX00166*	*Glomerale*	*Glomeraceae*	-	FR693470.1	98%
OTU34	*Rhizophagus irregulare VTX00114*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	KC708370.1	97%
OTU35	*Rhizophagus sp.VTX00113*	*Glomerale*	*Glomeraceae*	*Rhizophagus*	JX144125.1	96%
OTU36	*Acaulospora sp. VTX00028*	*Diversisporale*	*Acaulosporacea*	*Acaulospora*	EU332732.1	97%

Figure S4. Agglomerative hierarchical clustering (AHC) using unweighted pair-group average method and Bray-Curtis dissimilarity, shown in a dendrogram to present the relationship between different AMF communities. Communities from low, moderate and high contaminated sites were presented as LC, MC and HC, while communities associated with soil samples and roots sampes were presented as R and S.

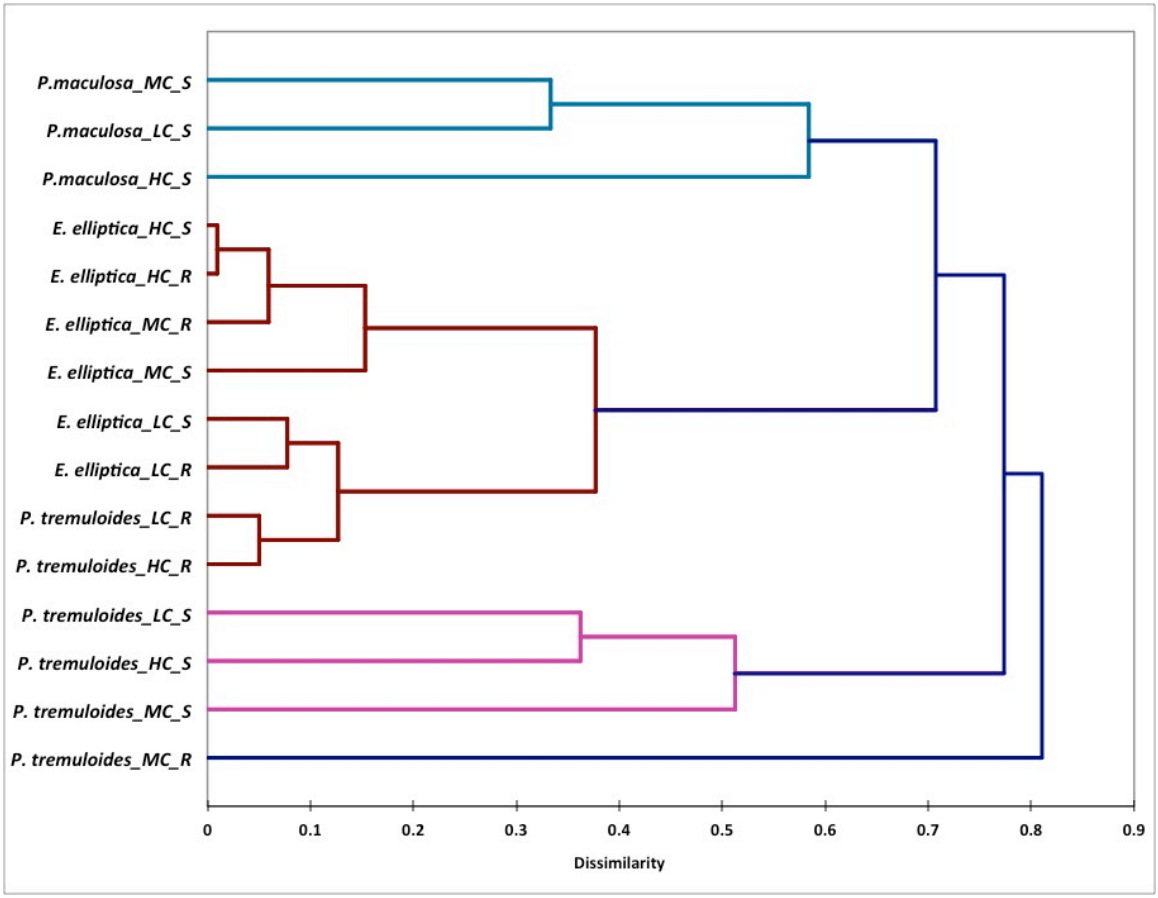

Table S5. Pearson correlation between AMF species and petroleum hydrocarbon chemicals in *E. elliptica* roots samples.

Eleocharise_root

Species/Hydrocarbons	*phenanthrene*	*acenaphrene*	*anthracene*	*fluorene*	*1-methylnaphtalene*	*1,3-Dimethylnaphtalene*	*H.P.*
Rhizophagus irregularis	0.504	0.542	0.495	0.546	0.548	0.514	0.444
Rhizophagus sp.	-0.126	-0.296	-0.111	-0.278	-0.266	-0.144	-0.043
Paraglomus sp.		-0.408	-0.442	-0.42	-0.427	-0.447	-0.422

Pearson corrlation metrics

Pearson's value was shown between each AMF species and each hydrocharbon pollutant.

*Significance value P<0.001 marked as ***; 0.001<P<0.01 marked as **; 0.01<P<0.05 marked as **.*

H.P. stands for total Petroleum Hydrocarbons (C10-C50).

Table S6. Pearson correlation between AMF species and petroleum hydrocarbon chemicals in *E. elliptica* soil samples.

Eleocharis_soil

Species/Hydrocarbons	*phenanthrene*		*acenaphrene*		*anthracene*		*fluorene*		*1-methylnaphtalene*		*1,3-Dimethylnaphtalene*		*H.P.*	
Rhizophagus irregularis	*0.045*		*-0.293*		*0.071*		*-0.252*		*-0.225*		*0.013*		*0.185*	
Claroideoglomus sp.1	***-0.687***	*	***-0.728***	*	***-0.676***	*	***-0.735***	*	***-0.738***	*	***-0.700***	*	*-0.609*	*P=0.081*
Rhizophagus sp.	*0.117*		*0.453*		*0.09*		*0.415*		*0.389*		*0.151*		*-0.036*	

Pearson corrlation metrics

Pearson's value was shown between each AMF species and each hydrocarbon pollutant.

*Significance value P<0.001 marked as ***; 0.001<P<0.01 marked as **; 0.01<P<0.05 marked as *.*

H.P. stands for total Petroleum Hydrocarbons (C10-C50).

Table S7. Pearson correlation between AMF species and petroleum hydrocarbon chemicals in *P. tremuloides* roots samples.

populus_root

Species/Hydrocarbons	***phenanthrene***	***acenaphrene***		***anthracene***	***fluorene***		***1-methylnaphtalene***	***1,3-Dimethylnaphtalene***	***H.P.***
Rhizophagus irregularis	*-0.185*	*-0.607*	*P=0.083*	*-0.149*	*-0.561*		*-0.528*	*-0.228*	*0.011*
Claroideoglomus sp.1	*0.176*	*0.366*		*0.159*	*0.346*		*0.333*	*0.196*	*0.08*
Acaulospora sp.	*0.181*	*0.581*		*0.147*	*0.537*		*0.506*	*0.221*	*-0.006*
Rhizophagus sp.	*-0.278*	*-0.628*	*P=0.07*	*-0.247*	*-0.592*	*P=0.093*	*-0.566*	*-0.315*	*-0.104*
Claroideoglomus sp.2	*0.073*	*0.321*		*0.053*	*0.293*		*0.274*	*0.098*	*-0.039*

Pearson corrlation metrics

Pearson's value was shown between each AMF species and each hydrocharbon pollutant.

*Significance value P<0.001 marked as ***; 0.001<P<0.01 marked as **; 0.01<P<0.05 marked as *.*

H.P. stands for total Petroleum Hydrocarbons (C10-C50).

Claroideoglomus sp.1 referes to as Claroideoglomus sp. VTX00193 ; Claroideoglomus sp.2 referes to as Claroideoglomus sp. VTX00276.

Table S8. Pearson correlation between AMF species and petroleum hydrocarbon chemicals in *P. tremuloides* soil samples.

Populus_soil

Species/Hydrocarbons	***phenanthrene***		***acenaphrene***		***anthracene***		***fluorene***		***1-methylnaphtalene***		***1,3-Dimethylnaphtalene***		***H.P.***
Rhizophagus irregularis	*-0.633*	*P=0.067*	***-0.766***	*	*-0.615*	*P=0.078*	***-0.761***	*	***-0.756***	*	*-0.654*	*P=0.056*	*-0.52*
Claroideoglomus sp.1	*0.142*		*0.273*		*0.13*		*0.26*		*0.251*		*0.157*		*0.074*
Acaulospora sp.	*0.103*		*0.454*		*0.074*		*0.415*		*0.387*		*0.138*		*-0.055*
Rhizophagus sp.	*-0.465*		*-0.492*		*-0.457*		*-0.497*		*-0.499*		*-0.473*		*-0.412*
Diversispora eburnea	*0.109*		*0.478*		*0.078*		*0.436*		*0.407*		*0.145*		*-0.058*
Claroideoglomus sp.2	*0.392*		*0.171*		*0.404*		*0.204*		*0.225*		*0.376*		*0.451*
Funneliformis mosseae	*0.392*		*0.171*		*0.404*		*0.204*		*0.225*		*0.376*		*0.451*
Diversispora celata	*0.073*		*0.321*		*0.053*		*0.293*		*0.274*		*0.098*		*-0.039*

Pearson corrlation metrics

Pearson's value was shown between each AMF species and each hydrocharbon pollutant.

*Significance value P<0.001 marked as ***; 0.001<P<0.01 marked as **; 0.01<P<0.05 marked as *.*

H.P. stands for total Petroleum Hydrocarbons (C10-C50).

Claroideoglomus sp.1 referes to as Claroideoglomus sp. VTX00193 ; Claroideoglomus sp.2 referes to as Claroideoglomus sp. VTX00276.

Chapter 3

Conclusions and Perspectives

In my master project, I assessed the richness of AMF associated with three plant species spontaneously growing in three sedimentation basins. In a total of 36 OTUs, 27 OTUs were singletons or doubletons while 9 OTUs were represented by more than two sequences and were used for in-depth analyses. The AMF richness found in these basins was in line with previous studies (Hassan et al. 2014; de la Providencia et al. 2015). We also showed that the community structure of arbuscular mycorrhizal fungi (AMF) was influenced by host plant identity, petroleum hydrocarbon contamination concentrations and biotopes (rhizosphere and roots). The shifts of the community structures of AMF could be explained by possible AMF-host preference versus specificity, biotopes, pollutant tolerance of AMF, and also by AMF taxa interactions.

A specificity or preference between AMF and their host plants has been previously reported in many studies (Sanders 2003, Montesinos-Navarro, Segarra-Moragues et al. 2012, Torrecillas, Alguacil et al. 2012). In my project, we found that some AMF OTUs were usually associated with certain host plants, such as *Acaulospora* sp. VTX00028, *Claroideoglomus* sp. VTX00193, *Claroideoglomus* sp. VTX00276, *Funneliformis mosseae* VTX00067, *Diversispora celata* VTX00060 that were more frequently found in *P. tremuloides* while *Paraglomus* sp. VTX00350 were highly associated with *E. elliptica.* These phenomenons reveal the unique biotic interrelationships between AMF and local plants under the petroleum contamination.

We found that *R. irregularis* was largely dominant in most of samples, but showed negative correlation with the petroleum hydrocarbon pollutants in *P. tremuloides* soil samples, which means the *R. irregularis* could be potentially influenced by the pollutant concentration.

The AMF community structure differences between plant roots and rhizospheric soil have been reported in many studies (Hassan et al. 2011, Hassan, Bell et al. 2014, de la Providencia, Stefani et al. 2015). Some AMF taxa tend to form mycelium inside of the plant roots and barely form

mycelium outside of the plant roots, thus are mostly found in roots while other taxa tend to produce massive mycelium outside roots, therefore can be mostly detected in the rhizospheric soil. Some AMF taxa can produce mycelium both inside and outside roots, so these taxa can be detected in both biotopes. In my study, I found negative correlations between AMF species and pollutants that were only observed in the soil samples.

The interaction between AMF, host plants and environment is much complex and dynamic. Further studies are needed to understand the basis of the molecular mechanisms of AMF involved in the degradation of organic pollutants and sequestration of inorganic contaminants. Many investigations on microbial communities have developed and tested synthetic communities on plant growth and protection against pathogen attacks. It would be interesting to study the effect and dynamics of synthetic AMF communities in phytoremediation applications and their role in yield increase and rehabilitation of perturbed sites, respectively. Finally, it is also important to investigate complex interactions of AMF taxa and other microbes including protozoa, algae and procaryotes, this could be a better way to understand the complete micro-interaction system.

References

Adesanya, O. A., L. A. J. Shittu, E. A. Omonigbehin and A. O. Tayo (2009). "Spermatotoxic impact of bonny light crude oil (BLCO) ingestion on adult male Swiss albino mice." International Journal of Physical Sciences **4**(5): 349-353.

Alkan, N., V. Gadkar, O. Yarden and Y. Kapulnik (2006). "Analysis of quantitative interactions between two species of arbuscular mycorrhizal fungi, Glomus mosseae and G. intraradices, by real-time PCR." Applied and Environmental Microbiology **72**(6): 4192-4199.

Alori, E. and O. Fawole (2012). "Phytoremediation of Soils Contaminated with Aluminium and Manganese by Two Arbuscular Mycorrhizal Fungi." Journal of Agricultural Science **4**: 8.

Alvarenga, P., A. P. Goncalves, R. M. Fernandes, A. de Varennes, G. Vallini, E. Duarte and A. C. Cunha-Queda (2009). "Organic residues as immobilizing agents in aided phytostabilization: (I) Effects on soil chemical characteristics." Chemosphere **74**(10): 1292-1300.

Amaya-Carpio L., F. T. D. J., M.A. Arnold (2005). "Arbuscular mycorrhizal fungi, organic and inorganic controlled-release fertilizers: Effects on growth and leachate of container-grown bush morning glory (Ipomoea carnea ssp. fistulosa) under high production temperatures." J. Amer. Soc. Hort. Sci.(130): 131-139.

Azaizeh, H. A., H. Marschner, V. Romheld and L. Wittenmayer (1995). "Effects of a Vesicular-Arbuscular Mycorrhizal Fungus and Other Soil-Microorganisms on Growth, Mineral Nutrient Acquisition and Root Exudation of Soil-Grown Maize Plants." Mycorrhiza **5**(5): 321-327.

Baker, J. M. (1970). "The effects of oils on plants." Environmental Pollution **1**(1): 27-44.

Barea, J. M., M. J. Pozo, R. Azcon and C. Azcon-Aguilar (2005). "Microbial co-operation in the rhizosphere." J Exp Bot **56**(417): 1761-1778.

Bell, T. H., S. E. Hassan, A. Lauron-Moreau, F. Al-Otaibi, M. Hijri, E. Yergeau and M. St-Arnaud (2014). "Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny." Isme Journal **8**(2): 331-343.

Borisev, M., S. Pajevic, N. Nikolic, A. Pilipovic, B. Krstic and S. Orlovic (2009). "Phytoextraction of Cd, Ni, and Pb Using Four Willow Clones (Salix spp.)." Polish Journal of Environmental Studies **18**(4): 553-561.

Brundrett, M. (1991). "Mycorrhizas in Natural Ecosystems." Advances in Ecological Research **21**: 171-313.

Cabello, M. N. (1999). "Effectiveness of indigenous arbuscular mycorrhizal fungi (AMF) isolated from hydrocarbon polluted soils." Journal of Basic Microbiology **39**(2): 89-95.

Cano, C. and A. Bago (2005). "Competition and substrate colonization strategies of three polyxenically grown arbuscular mycorrhizal fungi." Mycologia **97**(6): 1201-1214.

Colombo, R. P., L. F. Bidondo, V. A. Silvani, M. B. Carbonetto, N. Rascovan, M. J. Bompadre, M. Pergola, G. Cuenca and A. M. Godeas (2014). "Diversity of arbuscular mycorrhizal fungi in soil from the Pampa Ondulada, Argentina, assessed by pyrosequencing and morphological techniques." Canadian Journal of Microbiology **60**(12): 819-827.

Darriba, D., G. L. Taboada, R. Doallo and D. Posada (2012). "jModelTest 2: more models, new heuristics and parallel computing." Nature Methods **9**(8): 772-772.

Davies, F. T., J. R. Potter and R. G. Linderman (1993). "Drought Resistance of Mycorrhizal Pepper Plants Independent of Leaf P-Concentration - Response in Gas-Exchange and Water Relations." Physiologia Plantarum **87**(1): 45-53.

Davies, F. T., J. R. Potter and R. G. Linuerman (1993). "Drought resistance of mycorrhizal pepper plants independent of leaf P concentration - response in gas exchange and water relations." Physiologia Plantarum **87**(1): 45-53.
Davies, F. T., J. D. Puryear, R. J. Newton, J. N. Egilla and J. A. S. Grossi (2002). "Mycorrhizal fungi increase chromium uptake by sunflower plants: Influence on tissue mineral concentration, growth, and gas exchange." Journal of Plant Nutrition **25**(11): 2389-2407.
Davies, F. T., J. D. Puryear, R. J. Newton, J. N. Egilla and J. A. Saraiva Grossi (2002). "Mycorrhizal Fungi Increase Chromium Uptake by Sunflower Plants: Influence on Tissue Mineral Concentration, Growth, and Gas Exchange." Journal of Plant Nutrition **25**(11): 2389-2407.
de la Providencia, I. E., F. O. P. Stefani, M. Labridy, M. St-Arnaud and M. Hijri (2015). "Arbuscular mycorrhizal fungal diversity associated with Eleocharis obtusa and Panicum capillare growing in an extreme petroleum hydrocarbon-polluted sedimentation basin." Fems Microbiology Letters **362**(12).
Desjardins, D., W. G. Nissim, F. E. Pitre, A. Naud and M. Labrecque (2014). "Distribution patterns of spontaneous vegetation and pollution at a former decantation basin in southern Quebec, Canada." Ecological Engineering **64**: 385-390.
Edgar, R. C. (2004). "MUSCLE: multiple sequence alignment with high accuracy and high throughput." Nucleic Acids Research **32**(5): 1792-1797.
Eom, A. H., D. C. Hartnett and G. W. T. Wilson (2000). "Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie." Oecologia **122**(3): 435-444.
Estrada-Luna, A. A., F. T. Davies and J. N. Egilla (2000). "Mycorrhizal fungi enhancement of growth and gas exchange of micropropagated guava plantlets (Psidium guajava L.) during ex vitro acclimatization and plant establishment." Mycorrhiza **10**(1): 1-8.
Favas, P. (2014). Phytoremediation of Soils Contaminated with Metals and Metalloids at Mining Areas: Potential of Native Flora. Environmental Risk Assessment of Soil Contamination.
Garbisu, C. and I. Alkorta (2001). "Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment." Bioresource Technology **77**(3): 229-236.
Gerdemann, J. (1955). "Relation of large soil-born spore to phycomycetous mycorrhizal infections." Mycologia **47**: 619-632.
Govannetti, M. (1985). "Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune." Transactions of the British Mycological Society **84**: 679-684.
Guo, X. H. and J. Gong (2014). "Differential effects of abiotic factors and host plant traits on diversity and community composition of root-colonizing arbuscular mycorrhizal fungi in a salt-stressed ecosystem." Mycorrhiza **24**(2): 79-94.
Hassan, S. E., M. Hijri and M. St-Arnaud (2013). "Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil." New Biotechnology **30**(6): 780-787.
Hassan, S. E. D., T. H. Bell, F. O. P. Stefani, D. Denis, M. Hijri and M. St-Arnaud (2014). "Contrasting the Community Structure of Arbuscular Mycorrhizal Fungi from Hydrocarbon-Contaminated and Uncontaminated Soils following Willow (Salix spp. L.) Planting." Plos One **9**(7).

Hassan, S. E. D., E. Boon, M. St-Arnaud and M. Hijri (2011). "Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils." Molecular ecology **20**(16): 3469-3483.
Hijri, M. (2016). "Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield." Mycorrhiza **26**(3): 209-214.
Irwin, R. J. (1997). Environmental Contaminants Encyclopedia
Phenanthrene Entry, National Park Service.
Jacobson, J. L. and S. W. Jacobson (1996). "Intellectual impairment in children exposed to polychlorinated biphenyls in utero." N Engl J Med **335**(11): 783-789.
Jadia, C. and M. Fulekar (2009). "Phytoremediation of heavy metals: Recent techniques." African Journal of Biotechnology **6**: 921-928.
Jeffries, P., S. Gianinazzi, S. Perotto, K. Turnau and J.-M. Barea (2003). "The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility." Biology and Fertility of Soils **37**(1): 1-16.
Jiang, L. Y., X. E. Yang and Z. L. He (2004). "Growth response and phytoextraction of copper at different levels in soils by Elsholtzia splendens." Chemosphere **55**(9): 1179-1187.
Johansson, J. F., L. R. Paul and R. D. Finlay (2004). "Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture." Fems Microbiology Ecology **48**(1): 1-13.
Jones, R. K., W. H. H. Sun, C. S. Tang and F. M. Robert (2004). "Phytoremediation of petroleum hydrocarbons in tropical coastal soils - II. Microbial response to plant roots and contaminant." Environmental Science and Pollution Research **11**(5): 340-346.
Karami, A. and Z. H. Shamsuddin (2010). "Phytoremediation of heavy metals with several efficiency enhancer methods." African Journal of Biotechnology **9**(25): 3689-3698.
Kohler, A., A. Kuo, L. G. Nagy, E. Morin, K. W. Barry, F. Buscot, B. Canback, C. Choi, N. Cichocki, A. Clum, J. Colpaert, A. Copeland, M. D. Costa, J. Dore, D. Floudas, G. Gay, M. Girlanda, B. Henrissat, S. Herrmann, J. Hess, N. Hogberg, T. Johansson, H.-R. Khouja, K. LaButti, U. Lahrmann, A. Levasseur, E. A. Lindquist, A. Lipzen, R. Marmeisse, E. Martino, C. Murat, C. Y. Ngan, U. Nehls, J. M. Plett, A. Pringle, R. A. Ohm, S. Perotto, M. Peter, R. Riley, F. Rineau, J. Ruytinx, A. Salamov, F. Shah, H. Sun, M. Tarkka, A. Tritt, C. Veneault-Fourrey, A. Zuccaro, C. Mycorrhizal Genomics Initiative, A. Tunlid, I. V. Grigoriev, D. S. Hibbett and F. Martin (2015). "Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists." Nat Genet **47**(4): 410-415.
Kruger, M., C. Kruger, C. Walker, H. Stockinger and A. Schussler (2012). "Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level." New Phytologist **193**(4): 970-984.
Layton, A. C., J. Sanseverino, B. W. Gregory, J. P. Easter, G. S. Sayler and T. W. Schultz (2002). "In vitro estrogen receptor binding of PCBs: measured activity and detection of hydroxylated metabolites in a recombinant yeast assay." Toxicol Appl Pharmacol **180**(3): 157-163.
Lee, J., S. Lee and J. P. Young (2008). "Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi." FEMS Microbiol Ecol **65**(2): 339-349.
Lee, Y. J. and E. George (2005). "Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply." Plant and Soil **278**(1-2): 361-370.

Long, X., X. Yang and W. Ni (2002). "[Current situation and prospect on the remediation of soils contaminated by heavy metals]." Ying Yong Sheng Tai Xue Bao **13**(6): 757-762.
M. Zarei, S. K., S. Hempel, M.K. Nekouei, G. Savaghebi, F. Buscot (2008). "Community structure of arbuscular mycorrhizal fungi associated to Veronica rechingeri at the Anguran zinc and lead mining region." Environ. Pollut. **156**: 1277 -1283.
Ma, W. K., S. D. Siciliano and J. J. Germida (2005). "A PCR-DGGE method for detecting arbuscular mycorrhizal fungi in cultivated soils." Soil biology & biochemistry **37**(9): 1589-1597.
Magrou, M. (1936). Culture et inoculation du champignon de mycorhizes a arbuscules et a vesicules.
Moreira, I. T. A., O. M. C. Oliveira, J. A. Triguis, A. M. P. dos Santos, A. F. S. Queiroz, C. M. S. Martins, C. S. Silva and R. S. Jesus (2011). "Phytoremediation using Rizophora mangle L. in mangrove sediments contaminated by persistent total petroleum hydrocarbons (TPH's)." Microchemical Journal **99**(2): 376-382.
Moss, L. (2010). "The 13 largest oil spills in history." Retrieved July, 16, 2010, from http://www.mnn.com/earth-matters/wilderness-resources/stories/the-13-largest-oil-spills-in-history.
Opik, M., A. Vanatoa, E. Vanatoa, M. Moora, J. Davison, J. M. Kalwij, U. Reier and M. Zobel (2010). "The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota)." New Phytologist **188**(1): 223-241.
Ordinioha, B. and W. Sawyer (2010). "Acute health effects of a crude oil spill in a rural community in Bayelsa State, Nigeria." Niger J Med **19**(2): 140-144.
P. Cornejo, C. A.-A., J.M. Barea and N. Ferrol (2004). "Temporal temperature gradient gel electrophoresis (TTGE) as a tool
for the characterization of arbuscular mycorrhizal fungi." FEMS Microbiology Letters **241**: 265–270.
Pawlowska, T. E., R. L. Chaney, M. Chin and I. Charvat (2000). "Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill." Applied and environmental microbiology **66**(6): 2526-2530.
Prasad, M. N. V. and H. M. D. Freitas (2003). "Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology." Electronic Journal of Biotechnology **6**(3): 285-321.
Pulford, I. D. and C. Watson (2003). "Phytoremediation of heavy metal-contaminated land by trees - a review." Environment International **29**(4): 529-540.
Raskin, I. and B. D. Ensley (1999). Phytoremediation of toxic metals: Using plants to clean up the environment., John Wiley & Sons.
Reichenauer, T. G. and J. J. Germida (2008). "Phytoremediation of Organic Contaminants in Soil and Groundwater." Chemsuschem **1**(8-9): 708-717.
Reynolds, E. and M. Moslen (1977). "Damage to hepatic cellular membranes by chlorinated olefins with emphasis on synergism and antagonism." Environ Health Perspect **21**: 137-147.
Rivera-Becerril, F., C. Calantzis, K. Turnau, J.-P. Caussanel, A. A. Belimov, S. Gianinazzi, R. J. Strasser and V. Gianinazzi-Pearson (2002). "Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three Pisum sativum L. genotypes." Journal of experimental botany **53**(371): 1177-1185.
Robertson, L. W. and L. G. Hansen (1947). PCBs: Recent Advances in Environmental Toxicology and Health Effects, University Press of Kentucky.

Rossberg, M., W. Lendle and G. Pfleiderer (2006). Ullmann's Encyclopedia of Industrial Chemistry - Chlorinated Hydrocarbons.
Roy-Bolduc, A. and M. Hijri (2011). "The Use of Mycorrhizae to Enhance Phosphorus Uptake: A Way Out the Phosphorus Crisis." J Biofertil Biopestici **2**: 104.
Salt, D. E., M. Blaylock, N. P. B. A. Kumar, V. Dushenkov, B. D. Ensley, I. Chet and I. Raskin (1995). "Phytoremediation - a Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants." Bio-Technology **13**(5): 468-474.
Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn and C. F. Weber (2009). "Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities." Applied and Environmental Microbiology **75**(23): 7537-7541.
Schouteden, N., D. De Waele, B. Panis and C. M. Vos (2015). "Arbuscular Mycorrhizal Fungi for the Biocontrol of Plant-Parasitic Nematodes: A Review of the Mechanisms Involved." Front Microbiol **6**: 1280.
Smith and Read (2008). Mycorrhizal Symbiosis, Academic Press**:** 800.
Smith, S. E. and S. J. Barker (2002). "Plant phosphate transporter genes help harness the nutritional benefits of arbuscular, mycorrhizal symbiosis." Trends in Plant Science **7**(5): 189-190.
Sonjak, S., T. Beguiristain, C. Leyval and M. Regvar (2009). "Temporal temperature gradient gel electrophoresis (TTGE) analysis of arbuscular mycorrhizal fungi associated with selected plants from saline and metal polluted environments." Plant and Soil **314**(1): 25-34.
Stahl, J. D. (1949). "Die mycorrhiza de lebermoose mit besonderer beruchsichtigung der thallosen formen." Planta **37**: 103-148.
St-Arnaud M & Vujanovic V (2007) Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. Mycorrhizae in Crop Production,(Hamel C & Plenchette C eds.), pp. 67-122. Haworth Food & Agricultural Products Press, Binghamton NY.
Stewart, P., J. Reihman, E. Lonky, T. Darvill and J. Pagano (2000). "Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance." Neurotoxicol Teratol **22**(1): 21-29.
Su, Y. Y., X. Sun and L. D. Guo (2011). "Seasonality and Host Preference of Arbuscular Mycorrhizal Fungi of Five Plant Species in the Inner Mongolia Steppe, China." Brazilian Journal of Microbiology **42**(1): 57-65.
Susarla, S., V. F. Medina and S. C. McCutcheon (2002). "Phytoremediation: An ecological solution to organic chemical contamination." Ecological Engineering **18**(5): 647-658.
Torrecillas, E., M. M. Alguacil and A. Roldan (2012). "Host Preferences of Arbuscular Mycorrhizal Fungi Colonizing Annual Herbaceous Plant Species in Semiarid Mediterranean Prairies." Applied and Environmental Microbiology **78**(17): 6180-6186.
Velazquez, M. S., M. N. Cabello and M. Barrera (2013). "Composition and structure of arbuscular-mycorrhizal communities in El Palmar National Park, Argentina." Mycologia **105**(3): 509-520.
Wilde, P., A. Manal, M. Stodden, E. Sieverding, U. Hildebrandt and H. Bothe (2009). "Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes." Environmental microbiology **11**(6): 1548-1561.

Wu, F. Y., X. Z. Yu, S. C. Wu and M. H. Wong (2014). "Effects of Inoculation of Pah-Degrading Bacteria and Arbuscular Mycorrhizal Fungi on Responses of Ryegrass to Phenanthrene and Pyrene." International Journal of Phytoremediation **16**(2): 109-122.
Wu, L. Y., C. Q. Wen, Y. J. Qin, H. Q. Yin, Q. C. Tu, J. D. Van Nostrand, T. Yuan, M. T. Yuan, Y. Deng and J. Z. Zhou (2015). "Phasing amplicon sequencing on Illumina Miseq for robust environmental microbial community analysis." Bmc Microbiology **15**.
Xiang, D., E. Verbruggen, Y. J. Hu, S. D. Veresoglou, M. C. Rillig, W. Zhou, T. L. Xu, H. Li, Z. P. Hao, Y. L. Chen and B. D. Chen (2014). "Land use influences arbuscular mycorrhizal fungal communities in the farming-pastoral ecotone of northern China." New Phytologist **204**(4): 968-978.
Yang, H., Y. Zang, Y. Yuan, J. Tang and X. Chen (2012). "Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: evidence from ITS rDNA sequence metadata." BMC Evol Biol **12**: 50.
Yang, Y., Y. Liang, X. Han, T. Y. Chiu, A. Ghosh, H. Chen and M. Tang (2016). "The roles of arbuscular mycorrhizal fungi (AMF) in phytoremediation and tree-herb interactions in Pb contaminated soil." Sci Rep **6**: 20469.
Yang, Y. R., X. Z. Han, Y. Liang, A. Ghosh, J. Chen and M. Tang (2015). "The Combined Effects of Arbuscular Mycorrhizal Fungi (AMF) and Lead (Pb) Stress on Pb Accumulation, Plant Growth Parameters, Photosynthesis, and Antioxidant Enzymes in Robinia pseudoacacia L." Plos One **10**(12).
Yu, X. Z., S. C. Wu, F. Y. Wu and M. H. Wong (2011). "Enhanced dissipation of PAHs from soil using mycorrhizal ryegrass and PAH-degrading bacteria." Journal of Hazardous Materials **186**(2-3): 1206-1217.
Z. Liang, R. A. D., D.J. Lee, I.M. Dwiekat, S.D. Harris, D.A. Wedin (2008). " A DGGE-cloning method to characterize arbuscular mycorrhizal community structurein soil." Soil Biol. Biochem. **40**: 956 -966.
Zhang, H., Z. Dang, L. C. Zheng and X. Y. Yi (2009). "Remediation of soil co-contaminated with pyrene and cadmium by growing maize (Zea mays L.)." International Journal of Environmental Science and Technology **6**(2): 249-258.
Zhang, L., M. G. Xu, Y. Liu, F. S. Zhang, A. Hodge and G. Feng (2016). "Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium." New Phytologist **210**(3): 1022-1032.
Zhang, X., Y. Qu, Q. Ma, Z. Zhang, D. Li, J. Wang, W. Shen, E. Shen and J. Zhou (2015). "Illumina MiSeq Sequencing Reveals Diverse Microbial Communities of Activated Sludge Systems Stimulated by Different Aromatics for Indigo Biosynthesis from Indole." PLOS ONE **10**(4): e0125732.